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BULLETIN

OF THE

BUSSEY INSTITUTION

[JAMAICA PLAIN (BOSTON)]

VOL. III — PART I

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BULLETIN

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BUSSEY INSTITUTION.

No. 1. — *On the Power of some Peach Trees to Resist the Disease called "Yellows."* By E. W. Morse, Instructor in Natural History.

In the light of the knowledge concerning life processes that we now possess, the phenomenon known as "peach yellows" is manifestly a reaction of the peach tree tissues to some external influence. Inasmuch as this reaction is not normal to peach trees, it must be due to some weakness induced by an unfavorable environment either during the life of the individual plant in question or that of its ancestors. Since the trees which suffer from "yellows" usually show no sign of the disease in their youth (if it were otherwise they would never have been transplanted to a cultivated orchard), the only reasonable supposition is that they have been subjected to some unfavorable influence during the same generation in which the disease appears, or at the most in an earlier generation not very remote. In other words, a peach tree in which the malady occurs is, or has been, subjected to an environment differing from that of its ancestors, for otherwise the latter could not have survived to reproduce their kind.

Suggestions of external factors which might produce the disease have been many, but they may be classified as follows: —

(*a*) Microbes.
(*b*) Mechanical injuries.
(*c*) Improper soil conditions.
(*d*) Punctures of insects.
(*e*) Unsuitable climate.
(*f*) Enzyms.

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(*a*) Researches of cryptogamic botanists, particularly those of Dr. E. F. Smith, show that all cases of the disease cannot be caused by microbes, although the microbes are often present and may assist in weakening the host.

(*b*) (*c*) Investigations of Dr. Smith and others lead to the conclusion that, although mechanical injuries and improper conditions of the soil may aggravate the symptoms of the disease, neither of these accidents can be its primary cause.*

(*d*) Since diseases of a similar nature have been shown to be caused by animal parasites, such as aphides, mites and thrips on carnations,† and by mites on palms,‡ it has been suggested by Mr. Cook that possibly "yellows" may be caused by mites or other parasites. The probability of this view is strengthened by the fact that mites of the genus *Phytopetus* have frequently been found on the trees affected with the "yellows." It may well be that occasionally the injurious effects of this disease have been increased by the parasites; but as mites or other parasites are not found in all cases of the disease, this hypothesis leaves many cases unexplained, including those met with in the experience of Mr. Hersey, as will be stated further on.

(*e*) The idea formerly prevalent that the disease is due to climate has gradually been given up by most observers who have attempted to solve the problem, though, as will be shown directly, it is evident that certain phases of the subject have been overlooked, and that there are many facts on record which must be explained rationally before we can reach the conclusion that climate is not the predisposing cause of the disease. Among these facts are the following: —

(1) So far as I have been able to ascertain, peaches grown under glass are not affected.

(2) The disease has not appeared in California, in Europe, or in any country besides the United States.§

(3) Comparatively little harm has been caused by the "yellows" in the Southern States, or in States west of the Mississippi River.

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* Bull. No. 9, Bot. Div. U. S. Dept. Agr. (1888), and Bull. No. 4 (1893), Div. Veg. Path.

† Wood, Bull. 19, Div. Veg. Path. U. S. Dept. Agr. 1900.

‡ O. F. Cook, "Science," 1900, p. 875.

§ Bull. No. 1, Div. Veg. Path. U. S. Dept. Agr., 1891, p. 11.

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(4) Apricots, almonds, nectarines, peaches, and Japanese plums are, in the eastern American States, susceptible to attacks, while European and American plums are not.

(5) That at least one variety of peach which has been grown for over a hundred and fifty years in one locality here in Massachusetts and has become perfectly acclimated has never been affected, although surrounded by other varieties which were dying with the "yellows." From time to time there have been reports of varieties of peaches which have been resistant to the yellows. Careful investigation has usually shown, however, that sooner or later the so-called resistant variety has succumbed to the "yellows," or the variety has never been exposed to a case of genuine "yellows." What was supposed to be the "yellows" has usually proved to be a diseased condition due to nematodes, borers, or some other trouble. So commonly have these so-called resistant varieties failed that it has become customary to pay little attention to claims of this sort, or even to hold them in contempt. A Michigan peach grower expresses in the following words not only his own opinion in the matter, but that of the community at large: "People who claim that certain varieties are immune or less subject to 'yellows' than others belong to the same class that plant potatoes 'in the moon,' and believe pear blight to be occasioned by a spell of weather."

Here follows an account of the experience, in respect to peach "yellows," of my colleague, Mr. Edmund Hersey, and that of his ancestors, given in his own words:—

"Between 150 and 160 years ago a mariner, who sailed in a ship that transported fruit from Southern Europe to this country, gave Mr. Isaiah Hersey of Hingham, Mass., a few peach stones, which he planted in his garden. From these seeds he obtained three varieties of peaches. One was of good size, yellow flesh, free stone, and most excellent flavor, and was named the Hersey Rareripe. Another variety was of medium size, white flesh, free stone, and good flavor. This was named the White Magdalene. The third variety, which was named Mallacatoon, was a late peach of good size, white flesh, free stone, and of little value except for cooking.

"Seedling trees from the Hersey Rareripe would not always produce fruit exactly like that of the parent tree, although the variations were so slight that all of the peaches were very good. Seedlings from the Magdalene and the Mallacatoon always produced fruit of the same quality as that of the parent tree. Although planted in the vicinity of other varieties they have never been known to cross.

"When Mr. Reuben Hersey, the son of Isaiah, and the grandfather of the present writer, built his house, he planted seed obtained from his father's peach trees; and when his son, Samuel F., my father, built a house, he planted peach seed obtained from his father's peach orchard. In the spring, when I was four years old, my father staked out a small plot of land for me to use for growing such fruit and vegetables as I chose. My first effort was to grow a few peach trees from seed which my father had gathered from his peach trees the autumn before, and pressed into the ground to keep them in good condition until planting time the following spring. My first orchard had three peach trees in it, which commenced to produce fruit when they were four years old. Since that time I have been able to produce peaches every year without exception. My father's and my grandfather's houses are both between my house and that which was my greatgrandfather's. The distance between the two houses last named is less than sixty rods. These facts are given to show that seedling peaches from the same stock imported from Europe have been grown in the same locality for a period of more than one hundred and fifty years.

"One variety, the White Magdalene, remains strong and healthy, appearing to have become perfectly acclimated to this region. The other two varieties have dropped out, — one intentionally, and the other, the Hersey Rareripe, for want of information that has been very dearly bought. The details are as follows: A few years after the introduction of budded trees on stocks from seed grown in the Southern States (1845), the peach 'yellows' appeared in Hingham. I had been accustomed not to grow on my land any peach trees except from seed grown on my own seedling trees, and for many years abundant crops of peaches were borne on trees that were entirely free from the 'yellows.'

"One tree, of the Hersey Rareripe variety, grew within twenty-five feet of a neighbor's peach orchard, every tree of which, though dying with the 'yellows,' was permitted to stand until not a living sprout was left. So far as I was able to see, my tree, although more than thirty years old, was not in the least affected by any disease, so far as related to the wood. As this tree produced the best Rareripe peaches of any tree I had, for some years no other seed was planted except from this tree. After these seedlings had come into bearing, the favorite tree, which was parent of these seedlings, together with all other trees of the Rareripe variety planted before the 'yellows' were introduced, were destroyed to make room for building improvements. For several years the young orchard of seedlings from the favorite Rareripe produced large crops of excellent fruit. From this orchard one season I planted a peck of pits, and not one germinated. At first I thought the soil was unfavorable, but the next year I had no better success, although the seed was planted in various soils. Afterwards I planted large quantities of pits, but did not succeed in getting a single tree.

"A year or two after the seed failed to grow, I discovered that a few trees of this variety produced fruit that exhibited the first stages of the 'yellows.' The next year all of the oldest of these cross-fertilized offspring of the Rareripe stock began to send out very fine twigs, the leaves became yellow and the fruit ripened before fully grown, and some of it decayed on the tree. In a few years every tree died. Thus the variety called the 'Hersey Rareripe,' so far as I know, has been lost. Evidently, the pollen from diseased trees had affected the fruit of the sound trees, and trees growing from this infected seed were congenitally diseased.

"The White Magdalene, on the contrary, has withstood all the vicissitudes of our climate, and has never been known to cross with any other variety; it remains essentially the same peach as regards form, color, and quality that it was a hundred and fifty years ago. The seeds germinate as readily now as in former years. The twigs are large, ripen well, and are rarely killed by cold weather. No tree of this variety has ever shown the least indication of the 'yellows,' although some of them have been grown so near to trees that have died with the disease that the branches of the two sorts have intertwined. This experience leads to the conviction that the Hersey Rareripe might have been perpetuated if buds taken from trees grown from seeds produced before the introduction of the 'yellows' had been set in Magdalene stocks.

"As another item of experience I may mention the fact that nearly twenty years ago three trees grown from northern seed were budded with the Early Crawford peach. The buds all lived, and when two years old from the bud the trees were transplanted to the northern slope of a hill, the soil of which was a light sandy loam. The trees grew, and for five years produced a good crop of excellent peaches, though after that time the 'yellows' appeared on each tree. To observe the progress of the disease no limbs were cut off, but all were left to decay on the trees. The second year after the 'yellows' appeared I observed that a sprout had commenced to grow near the roots of each tree, and below where buds of the Early Crawford had been set. The sprouts made a very good growth considering the nature of the soil, and the fact that no fertilizer had been applied. The second year the limbs from the sprouts were up near the centre of the trees, and were surrounded by branches of the Early Crawford, dying with the 'yellows'; not a limb of the Crawford was cut off, but left to decay and fall to the ground, which they did in a few years, leaving the trees coming from the sprouts of the natural trees master of the situation. One of these trees, which I have left standing that I might continue the experiment, is now a good shaped tree; it produced a full crop of peaches last year, notwithstanding the peach crop, in Hingham, was a failure.

"From the above facts I am led to draw the inference that at least one variety of peach trees is not subject to the 'yellows.' Moreover, a careful review of my experience with seedling peach trees, many generations of which have been grown in one locality, leads to the conclusion that they become strong enough to prevent their wood from being contaminated by the 'yellows' from trees suffering from this disease only a few feet distant from them, though they may not in all cases become strong enough to prevent the pollen from trees having the 'yellows' affecting the seed coming from such crossing.

"As the peach tree is a native of a warmer climate than ours, only the most hardy varieties would be likely to succeed here. This view of the matter has not been taken by our peach growers who are accustomed to plant pits or trees that have been brought from Southern States. The change of climate creates a weakness which invites the 'yellows' and other troubles."

Mr. Hersey's idea as to the influence of climate in predisposing a plant to disease is presumably correct, for any marked change in the environment of an organism can hardly fail to bring about some change in its life processes, — a great or sudden change in the one causing a corresponding change in the other. All varieties of trees which have had the "yellows" were formerly grown in a different climate (usually warmer) than that in which the disease has appeared.

The introduction of plants or trees, like the peach and the nectarine, which are accustomed to a warm and equable climate, to one so changeable as that of Eastern North America, must inevitably result in physiological changes so great that in many instances a diseased condition is manifested. Some varieties would be disturbed more than others; also some individuals more than other individuals of the same variety. Still others, though slightly disturbed at first, might gradually recover their equilibrium, — that is to say, become acclimated. Only those individuals which could adapt themselves to their changed environment could survive for any length of time. Such individuals we call "hardy," or refer to them as having more "constitutional vigor," — terms more or less vague, but nevertheless convenient until our knowledge of such characters becomes more accurate, — by which is meant that they inherit certain characters which tend to enable them to continue in their life-work and remain like their ancestors in spite of the vicissitudes of external change and the consequent inducement to variation. Such characters the White Magdalene Peach seems to have, and the question arises why have not more varieties of peaches become acclimated? In the first place, it would appear that since the importance of the idea above stated has not been generally recognized hitherto or clearly grasped, no special pains have been taken to put it in practice. Because of this inattention, it is not improbable that many a tree which had become fairly well acclimated has been ignorantly destroyed.

Then, again, it is well known that the peach and some of its closely related species are not overburdened with those qualities included under the term "hardiness." They have, as a rule, just the opposite qualities which collectively may be called "tender." This is shown in many ways, — one notable instance is that in spraying peach trees to repel the attacks of insects and fungi, weaker solutions of chemicals must be used than are suitable for spraying many other plants. For this reason, many varieties or individuals might find it more difficult to become acclimated than if the peach and its relatives were more hardy. And it may be true that there are already in existence more acclimated varieties than have yet been discovered by the scientist. The discouragement caused by negative results in the past should not lead too easily to the impression that positive results are unattainable. Further investigation may lead to their discovery.

The observations of Mr. Hersey indicate the probability that many other farmers may have had similar experiences to his. Thus, upon a farm in southern New Hampshire, owned by the writer of this article, there are some twenty peach trees, and most of the neighbors have at least that number and some of them many more than twenty. Since I have been acquainted with that locality, — eleven years, — although we have had other infirmities of the peach tree, no case of "yellows" has appeared upon my farm or upon those of my neighbors, as far as I know. The trees appear to be acclimated, but whether or not they are sufficiently so to be able to survive if the "yellows" appeared in the vicinity, as has the Magdalene, no one is at present able to say. That Mr. Hersey has had on his land cases of genuine "yellows" there can be no question. One reason why this variety has become so well adjusted to its present home is the fact that it has been grown so long in the same locality. Having obtained a foothold and become adapted to a particular climate and soil, the internal functions have had time to adjust themselves to the external conditions presented to them. This method of procedure is contrary to that usually practiced by our peach growers; the peach pits planted have come from trees grown in the South, which are thus subjected to a new and violent change, in addition to that which the parent tree experienced when first imported from its foreign home. Thus, for a second time, have the normal functions been disturbed. This process when repeated several times, as it often has been, can hardly fail to affect any plant not having the qualities which give to it the characteristic of "hardiness," and which are so conspicuously lacking in the peach. Each change requires a readjustment to new conditions, with the result that before the tree has had time to accustom itself to a certain soil and situation it is removed to another situation, rendering it in the meantime extremely susceptible to infirmities of all kinds.

It is easy to suggest other reasons why the Magdalene has obtained so firm a hold. For instance, with our variable climate, the first season after its importation, and possibly the second, may have been milder or more even, and thus allowed more time for adjustment before unfavorable seasons were forced upon it. Again, the soil on which it was grown was not rich, and, unless heavily fertilized, less growth would be made each year than in a more fertile soil, thus increasing the probability of a better "ripening" of the wood to withstand the cold blasts of winter, — a factor now recognized among our peach growers, who try to force most of the growth of the twigs early in the season.

The experience of Mr. Hersey, together with the facts previously stated, show that climate is more or less a factor in the question as to the cause of peach "yellows"; yet experiments have shown that in some instances the disease may be communicated from one tree to another by budding, and by using tools in pruning a healthy tree after having come into contact with diseased tissues. Hence there must be some other factor not yet discussed; and we now turn to the last item in the list of suggestions on page 1, entitled (*f*): for, since the "yellows" behaves like an infectious disease and as experimental evidence has shown that it is not due to living organisms, the only alternative is that of enzyms.*

The probability that enzyms may be the true cause is further enhanced by the discovery of Buchner† that alcoholic fermentation set up by the yeast plant is due to an enzym which he has separated from it. Enzyms are normally present in plants and animals, but being extremely sensitive to environment they may become abnormally active under certain conditions not as yet well understood, and may be produced in large quantities. Wood* has shown that the oxidases and peroxidases are capable of decomposing chlorophyll when especially active, resulting in the variegations of the green parts of plants. The mosaic disease of tobacco he was able to produce at will by increasing the action of these enzyms. Beijerinck† also induced albino diseases by inoculating healthy plants with the filtered juice of spotted ones, and concludes that the mosaic disease is due to an enzym contained in a "contagium fluidum vivum."

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* Enzyms are secretions of protoplasm which provoke alterations (known as fermentations) in the cell contents, but do not usually enter into the changes themselves. They are classified according to the nature of the changes invoked, those causing oxidation are known as oxidases and peroxidases. For a further discussion of the nature and work of enzyms, see J. R. Green, "The Soluble Ferments and Fermentations," University Press, Cambridge, London, 1899; J. Effront, "Les Enzymes et leurs Applications," Carré et Naud, Paris, 1899.

† Berl. Ber. 1897, **30.** pp. 117, 1110.

It has often been suggested that "yellows" is a disease allied to variegation often found in other plants, and undoubtedly it is. According to Darwin,‡ variegation seems to result from a feeble and atrophied condition of the plant, and a large proportion of the seedlings raised from parents, both of which are variegated, usually perish at an early age. The first indication that anything was wrong with the Hersey Rareripe was that the seeds failed to germinate. It is also well known that variegated plants if grafted on green ones may induce the development of variegated leaves on both stem and stock, and that variegation may be inherited through the seed. Darwin cites many instances of the peculiar constitutional variations of the peach. Oxydized enzyms being present in normal peach trees, any slight change in the environment might incite them to greater activity and a consequent destruction of chlorophyll.

The objection which has always been brought against the enzym theory has been that the method by which the irritated enzym is carried from tree to tree is unknown. That stocks budded from diseased trees, and tools used in pruning might transfer the enzyms is easily conceivable, but any proof as to the contamination of seedling trees has been lacking until the history of the Hersey Rareripe Peach was known and the evidence carefully weighed. The evidence shows that the enzyms were carried from tree to tree in the pollen. That the disease is communicated in this way was suggested as long ago as 1828 by William Prince,§ who says of the "yellows": "This disease is spread at the time when the trees are in bloom, and is disseminated by the pollen or farina blowing from the flowers of the diseased trees and impregnating the flowers of those which are healthy, and which is quickly circulated by the sap through the branches and fruit, causing the fruit, wherever the infection extends, to ripen prematurely." Professor Smith evidently failed to appreciate the importance of this observation, and he has remarked that Prince "does not always distinguish clearly between things proved and things probable." Downing,* in his time, has said: "Experiments have been made by dusting the pollen of diseased trees upon the blossoms of healthy ones without communicating the 'yellows.'"

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* Centbl. Bak. 1899, V, p. 745.

† Botanisches Centralblatt, **78.** (1899) No. 5, pp. 146–151, abstracted from Separate Verhandlang K. Akad. Wetensch, Amsterdam, 1898.

‡ Animals and Plants under Domestication, Vol. II, p. 205.

§ A Short Treatise on Horticulture.

The inferences which were drawn from the experiments referred to by Downing are as onesided as are those of Mr. Prince. They show that Prince was wrong in inferring that the material in the pollen was quickly circulated by the sap and affected parts of the plant other than the impregnated ovule. Not until the attempt had been made to grow trees from such ovules was it safe to conclude that the disease was not carried by the pollen. With the Hersey Peach this experiment was carried out, and it shows that, although the disease was carried by the pollen, it was not communicated to the parent plant but affected the ovule only. Further evidence that pollen is the carrier of the disease, lies in the fact that only those trees whose ovules were susceptible of being fertilized with pollen of other varieties were attacked by the disease, while the White Magdalene which is sterile to foreign pollen has not been infected.

It may be suggested by some that not enough infecting material could be carried by such small bodies as pollen grains, but only a very small amount of infecting material is necessary; and Smith† also says: "Only a small amount of infective material is necessary, provided it be in the form of living cells, which can be induced to unite with the actively growing tissues of the tree." And on the same page he says: "Since diseased trees have been shown to be very full of infectious matter, it must be that for unknown reasons much of this fails to find an immediate entrance into healthy trees. Otherwise the peach would soon disappear entirely." This "unknown" reason appears to be that the disease is communicated in nature only by means of pollen, yet he seems to think infection does not take place through the blossoms, — a view which cannot now consistently be taken. The evidence shows that the disease is of the nature of a variegation due to a weakened constitution (brought about by an unfavorable climate) acted upon by enzyms which in nature are carried by the pollen from tree to tree. If the ovule is fertilized by the enzym-containing-pollen, the enzyms may either affect the germinating capacity of the seed or remain dormant for a period, until some unfavorable environment of the tree starts them into activity and an ordinary outbreak of "yellows" is manifested. Under domestication the "yellows" are propagated by planting seedlings so affected, or if sound seeds are planted the enzyms are transferred to the healthy stock by means of budding, and the disease is perpetuated. Downing* insisted that the "yellows" was in the nature of a constitutional taint, but no heed was paid to his warning. It was the custom of the nurseryman to gather the stones indiscriminately for stocks.

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* Fruit and Fruit Trees of America, p. 463.

† Bull. 1, Div. Veg. Path. U. S. Dept. Agr., p. 44.

The practical conclusion which may be drawn from this experience is that, to avoid the "yellows" as well as other diseases, we must look to the internal qualities of the stock — as has been done with many other plants — rather than to external remedies, or to the enacting of laws for the suppressing of symptoms or "gross appearances" of the disease. We might profit by the advice given long ago by Lindley and quoted by Downing* : "All seeds will not equally produce vigorous seedlings; but the healthiness of the new plant will correspond with that of the seed from which it sprang. For this reason it is not sufficient to sow a seed to obtain a given plant; but in all cases where any importance is attached to the result the plumpest and healthiest [and most thoroughly acclimated] seeds should be selected, if the greatest vigor is required in the seedling; and feeble or less perfectly formed seeds, when it is desirable to check natural luxuriance." This advice is now being followed, at least in some localities. According to Hon. Charles W. Garfield, of Michigan, since the appearance of the scourge of "yellows" in that State peach growers are more careful to select stock which is sound, and has not been grown where "yellows" is known to prevail. By this method of selection there is a greater probability of obtaining stock which has become acclimated.

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* Fruit and Fruit Trees of America, pp. 464, 465.

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No. 2. — *Testing for Mannose.* By F. H. Storer, Professor of Agricultural Chemistry.

It is commonly stated in the text-books * that, thanks to the sparing solubility of mannose-hydrazone in water, mannose may be detected readily, and even be determined quantitatively, by adding phenylhydrazin to its aqueous solution. In like manner, it is said that mannan may easily be detected and determined by boiling in a dilute acid the substances which contain it and adding phenylhydrazin to the liquid, after the acidity has been neutralized.

These statements are true enough in respect to the detection of mannose in solutions of this substance that are neither too dilute nor too much contaminated with impurities such as may result from the solvent action of water and acids on vegetable matters. They are true, also, in some part, in respect to substances that contain a sufficiently large proportion of mannan to override obscurations which impurities and other complications might induce. It is easy, for example, to detect mannose in solutions prepared by oxidizing mannite, and in those obtained by hydrolyzing date stones, vegetable ivory, or the wood of various coniferous trees. But to detect mannan in cases where but little of it is present, or, still worse, to prove that it is absent in any given instance, is a much more difficult task, as, for example, when the wood of deciduous trees is tested.

In the hope of gaining a proper mastery of the subject, I have had a very large number of tests for mannose made under my eye during the last few years, and have become so familiar with the difficulties which stand in the way of proving the absence of this substance that some part of the accumulated experience seems worthy of being put upon record.

In testing substances that contain an abundance of mannan, the presence of this substance may be demonstrated readily enough by boiling some of the powdered wood or other material together with ten or twelve times as much hydrochloric acid of five per cent. over a free flame during several hours in a flask provided with a reflux condenser; then neutralizing the acid liquor with dilute caustic soda, using phenolphthalein as an indicator if the solution is clear enough, and adding to the neutralized liquor either a mixture of phenylhydrazin and glacial acetic acid or of phenylhydrazin hydrochlorate and sodium acetate, or simply, though on the whole less conveniently, a solution of phenylhydrazin in water. Ordinarily, the reagent first named has been prepared by shaking together 1 cc. of phenylhydrazin, 2 cc. of glacial acetic acid, and 10 cc. water; and the second solution has been made by mixing the two constituents in molecular proportions. Usually 0.5 grm. of phenylhydrazin hydrochlorate is dissolved in 5 cc. of water and 0.3245 grm. of sodium acetate is added.

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* Cf. Tollens, Handbuch der Kohlenhydrate, **2.** 113; Maquenne, Les Sucres, Paris, 1900, p. 560.

The yellowish-red precipitate that soon falls on the addition of the phenylhydrazin reagent should be examined under the microscope from time to time, and, subsequently, for the sake of obtaining more perfect crystals, it should be washed with water and recrystallized from alcohol. As was noticed long ago by Fischer,* the yellowish color of the original precipitate is not in the least an indication of the presence of mannose, for mannose-hydrazone is almost absolutely colorless when pure. Usually the yellow precipitate will be seen under the microscope to contain considerable quantities of amorphous matter. At the best, the mannose-hydrazone will appear in the form of bunches of radiating prismatic crystals with blunt ends, — that is to say, the terminations of the crystals are chamfered or cut off at a bevel; they have no sharp needle-like points nor are the ends of the crystals actually square or rectangular. But it often happens that mannose-hydrazone, when first precipitated from the neutralized product of the hydrolysis of vegetable matters, takes the form not of crystals but of smooth globules of greenish-yellow or brownish-yellow color. These balls are not perfectly round, as a general rule, and they are apt to be joined one to another more or less closely. They resemble somewhat in appearance the large, round cells and clusters of cells of some varieties of yeast. Not infrequently the globules are no longer smooth but beset with numberless very short points, as of needles about to shoot forth. Sometimes mixtures of smooth globules, roughened globules, and the radiating crystals with blunt ends appear simultaneously. Speaking in very general terms, it may be said that the "original precipitate" which appears on adding the phenylhydrazin reagent to solutions of mannose consists more frequently of the smooth globules than of the crystalline forms, though the latter may be exhibited by dissolving the globules in alcohol and allowing the solution to crystallize. As will be stated below, there are several kinds of globules which may appear in the "original precipitate," but it is none the less true that the globules of mannose-hydrazone exhibit certain peculiarities which appeal more or less strongly to an experienced eye; they are apt, moreover, to appear soon after the addition of the reagent, unless the mannose solution was very dilute. It is essential, however, in every instance to convert the globules to crystals.

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* Tollens, Handbuch der Kohlenhydrate, **2.** 115.

The thought that the prismatic crystals might perhaps result from the crystallization of the globules as they lie quietly in the midst of the original precipitate, — that is, from a rearrangement of the particles of matter of which the globules are composed, even though no re-solution of them had occurred, — has not been borne out by experience. This idea seemed at first to be strengthened in some measure by the occasional formation upon the globules of the fringe of crystalline points, above mentioned, which gives the globules a roughened appearance. Sometimes, it has almost seemed as if the contents of the smooth globules might possibly be in a condition akin to that of a supersaturated saline solution almost but not quite ready to crystallize, but on crushing some of the globules under the microscope they fell to an amorphous powder; though, on the other hand, on crushing some of the roughened globules, on which short points or spines of projecting crystals had appeared, it has been noticed once or twice that they exhibited a crystalline structure internally, in that they consisted of a bundle of minute fibrous crystals radiating from a point at the centre of the ball. Occasionally the short, blunt spines springing from the yeast-like globules show distinctly that they are really the bevelled terminations of prismatic or plate-like crystals lying in bundles within the ball. Generally speaking, the appearance of mannose-hydrazone in either of the forms above described is sufficiently characteristic, especially to any one who has had some experience in testing for this substance, though a beginner might perhaps do well to prepare a small quantity of mannose expressly by hydrolyzing date stones or the ivory nut, and comparing the reactions of this substance with those exhibited by a suspected material.

To make assurance doubly sure, it is well to recrystallize from warm diluted alcohol the mannose-hydrazone that was first thrown down, and to view the new crop of crystals under the microscope. By so doing, the globules of mannose-hydrazone may be changed to the highly characteristic crystals with bevelled ends, and a cleaner field be obtained also for viewing the crystals. One constant trouble, for instance, in testing for mannose, even in materials that are known to be unusually pure, is the formation of the above-mentioned fine amorphous yellow powder on the addition of the phenylhydrazin reagent. Since it might readily deceive the uninstructed or unaided eye, it is absolutely necessary to examine the precipitate with the microscope in order to be sure that globules or crystals of mannose-hydrazone are contained in it. It seems probable that some part of this yellow dust may be formed by the union of phenylhydrazin with an oxidation product of dextrose. It may not be connected in any way with the presence of mannose, for it forms readily in solutions that contain no mannose. Even aqueous solutions of pure dextrose give faint yellow precipitates on being tested with phenylhydrazin, as will be set forth directly. Happily, this yellow dust is soluble in glacial acetic acid, and it may be removed from the field of the microscope by applying a drop of this acid.

For testing the various points above mentioned a quantity of a solution of tolerably pure mannose was prepared by hydrolyzing vegetable ivory with sulphuric acid, and numberless experiments were made by taking measured quantities of the standard solution thus obtained, diluting them with varying quantities of water and adding to definite volumes of these solutions stated numbers of drops of one or another of the phenylhydrazin reagents, taken in varying degrees of concentration and applied in such manner that sometimes the phenylhydrazin and sometimes the mannose should be in excess. The results of these trials showed that tolerably concentrated solutions are needed in order that satisfactory results may be obtained. If the solution of mannose is too dilute, or, in general, if too small a quantity either of mannose or of phenylhydrazin is present, no good results are to be expected. But in somewhat stronger solutions precipitates composed of yeast-like globules or of globules with stubby spines or of crystalline bundles composed of rhombic prisms will appear.

It should be understood clearly that the mere formation of a yellowish precipitate, on adding phenylhydrazin compounds to products of the hydrolysis of wood or other vegetable matter, is of itself no evidence whatsoever of the presence of mannose in those liquors, for yellowish or yellow-red precipitates may be produced by phenylhydrazin under a great variety of circumstances, — notably in most solutions obtained by hydrolyzing vegetable matters, in solutions of dextrose, as has been said, and also when a dilute aqueous solution of tannic acid is mixed with phenylhydrazin acetate. Even when distilled water, phenylhydrazin, and acetic acid are mixed, a slight turbidity appears at once, and a visible yellowish amorphous deposit will separate in the course of a day or two.

Recrystallization of Mannose-hydrazone from diluted Alcohol.

As a preliminary to the recrystallization of the mannose-hydrazone from alcohol, the liquid lying above the original precipitate should be removed either by decantation or by means of a small pipette or by absorption with little rolls of filter paper; the precipitate should be washed with water, by decantation, to remove sodium chloride, etc., and finally be covered with diluted alcohol (three parts of alcohol of 95 per cent. and one part of water) and warmed on a water bath to 75 or 80° C. The alcoholic solution is then allowed to evaporate very slowly until crystals appear. In case suspicion should arise in any instance that the yeast-like globules of mannose-hydrazone above described might possibly be not mannose-hydrazone but simply small drops of free phenylhydrazin, the doubt may be dispelled readily by treating the washed precipitate with a little glacial acetic acid, which will dissolve phenylhydrazin and other matters, but not the mannose-hydrazone. It is to be noted that the glacial acid should be applied to the washed precipitate, for if it were put upon a precipitate contaminated with sodium chloride, some of the latter might be precipitated in crystalline forms. Even on allowing a drop of glacial acetic acid to flow in upon well-washed balls beneath the cover glass on the slide of the microscope, extraneous matters may be removed, and, in case the mannose-hydrazone balls had a reddish tinge, the acid will remove this coloration and leave them either colorless or pale yellow.

By applying the alcohol in the manner above described, it is easy to obtain excellent crystals of mannose-hydrazone that leave no shade or suspicion of doubt as to their identity. They appear as colorless, brilliant rhombic prisms, sometimes as distinct plates, often as bunches of rhombic prismatic crystals radiating from a central point, and occasionally (when the alcohol has evaporated too rapidly) as a multitude of small stars spread like a carpet across the field of the microscope, with the points of each star touching those of its neighbors. Although this star carpet is an object of great beauty, its appearance is not to be encouraged, since the stars are not specially characteristic of mannose-hydrazone. In case the liquor resulting from the hydrolysis of the wood or other matter to be tested should be very dark colored, it might be decolorized by means of bone-black after neutralization and before testing with the phenylhydrazin reagents, but in many cases this complication is unnecessary.

It is not strictly necessary that the acid product of the hydrolysis of vegetable matters should be neutralized exactly by the soda lye before adding the phenylhydrazin reagent. Good results may still be obtained when the point of neutrality has been overstepped and the solution made faintly alkaline, and also when the liquor has been left slightly acid. In case phenylhydrazin acetate is to be used for testing, it has been found convenient to make the liquor from the hydrolysis very slightly alkaline, while, if phenylhydrazin hydrochlorate plus sodium acetate is to be used, it will be well enough to leave the liquor faintly acid. Perhaps rather better results, on the whole, are obtained by testing with phenylhydrazin acetate than with the mixture of phenylhydrazin hydrochlorate and sodium acetate, though when this last named mixture is used there seems to be a smaller liability that the reaction may be obscured after a time by the formation of crystals of dextrose-osazone.

The details above mentioned have been tested in this laboratory in a multitude of instances and in many different ways by operating upon vegetable ivory, date stones, the wood of coniferous and deciduous trees, etc., and with mannose prepared expressly for the purpose by the oxidation of mannite. They have been applied, moreover, not only to cases where hydrochloric acid has served as the hydrolyzing agent, but also to numerous instances where the woods have been treated with sulphuric acid of various degrees of concentration, and where preparations of barium and calcium carbonates of divers kinds have served as neutralizing agents, — a method, by the way, much less speedy and less convenient for the purposes of this research than the use of dilute hydrochloric acid, as above set forth.

As a fairly characteristic example of the behavior of the mannose-hydrazone precipitate, the following results may be cited. From a stock of air-dried mannose-hydrazone, prepared from mannite seven months previously and kept in a tightly closed bottle, several small parcels were taken and warmed upon a water bath to 85° or 90°, with smaller quantities of 4 : 1 alcohol than were needed for complete solution, and the clear solutions were decanted into watch-glasses. As the alcohol cooled, yellow precipitates separated which were seen under the microscope to consist of globules, some of them joined symmetrically so as to form rosettes (tetrads). On filtering the alcoholic solutions, to make sure that every particle of the undissolved hydrazone should be removed, a slightly different result was obtained in that, beside the yeast-like globules there were now to be seen a number of rather poor specimens of small crystalline balls covered with broad, blunt spines. On trying again to change the yeast-like balls to proper crystals, all the above mentioned separate parcels of materials were put together and warmed with diluted alcohol (4 : 1) until almost the whole of the hydrazone had dissolved. The decanted liquor was then allowed to evaporate very slowly. The yellow sediment showed under the microscope many smooth globules and some with broad spines. On repeating the trial and allowing the alcoholic solution to evaporate still more slowly in a cold room, a similar result was obtained. But on starting anew with some of the same, original, air-dried, finely powered hydrazone and heating it to 80° or 85°, with a mixture of three parts of alcohol of 95 per cent. and one part water, the decanted liquor deposited on cooling an abundance of true crystals, many of which were separate, individual, transparent, rhomboidal plates, while many others were plates radiating from a common centre, all showing the characteristic bevelled ends. On recrystallizing this material a second and a third time from the warm 3 : 1 alcohol, the same results were obtained. This work, it should be said, was done in the middle of December, — in cold, winter weather. It well illustrates the fact that occasionally the mannose-hydrazone balls are little disposed to change to the form of crystals. But, curiously enough, in another set of trials made five months afterward, in the middle of May, — in precisely the same way as before, in so far as could be seen, — very different results were obtained, and it was now found to be as difficult to get specimens of the globules, for exhibition under the microscope, as it had previously been to get crystals. The May sample was part and parcel of the same material as that used in December. Both samples were taken from the self-same bottle, which had stood quietly upon a shelf ever since the material was made, and, since the laboratory was kept at very much the same temperature in May as in December, it is not easy to believe that the different behavior of the two samples could have depended on the weather. At all events, on dissolving the May sample in 4 : 1 alcohol, at 85° to 90°, there was obtained, in repeated instances, excellent crystals of mannose-hydrazone, as the solutions cooled, but no globules at all. On using undiluted alcohol (of 95 per cent.) only crystals were obtained, though they were rather poor specimens. On using 3 : 1 alcohol, good crystals were obtained but no balls. With 2 : 1 alcohol excellent crystals separated on allowing the solution to evaporate slowly, and with 1 : 1 alcohol small but well-shaped crystals were obtained. From mixtures of one part alcohol and two parts water, and one alcohol to three water, some fairly good crystals and mixtures of crystals and roughened globules were got; and, finally, on using a mixture of one part alcohol and four parts water, an abundance of smooth, yeast-like globules separated, together with a few globules bearing stubby, broad spines.

In a number of instances experiments were made to test the question whether it might not perhaps be better to substitute acetone for the diluted alcohol used as above for purposes of recrystallization, but no evidence appeared that there would be any advantage in so doing. Attempts were made also to employ benzyl-phenylhydrazin, in alcoholic solution, for precipitating mannose, instead of the simple phenylhydrazin reagents above described, but the results of these trials gave no encouragement to proceed with them.

In Testing for Mannose, in products of hydrolysis, allowance must be made for the ultimate appearance of crystals of Dextrose-osazone.

In studying the composition of woods, the precipitation and recrystallization of mannose-hydrazone as above described would be a fairly satisfactory test in solutions of adequate strength, were it not for the circumstance that when dextrose is present — and a certain quantity of dextrose must necessarily appear whenever wood or any other substance containing cellulose is hydrolyzed — there will be formed, on the addition of the phenylhydrazin reagent, a quantity of soluble dextrose-hydrazone, some part of which will on standing be oxidized sooner or later to dextrose-osazone, the crystals of which might readily confuse or even mislead an inexperienced seeker of mannose; for this dextrose-osazone is a yellow precipitate, very difficultly soluble in water, which, under the microscope, is often seen to be composed of masses of crystals in the shape of balls, covered with numberless sharp-pointed spines or needles, which recall forcibly the appearance of the common sea-urchin (*Echinus*). Although to an experienced eye these "sea-urchins" are seen usually to differ not a little from the mannose-hydrazone balls covered with blunt crystals, as above mentioned, there are occasions or circumstances where the resemblance between the two species is really very close, and, as a general rule, the similarity is sufficient to confuse an uninstructed person. In point of fact, the liability of dextrose-hydrazone to be oxidized to an osazone on standing for any length of time in the air in contact with phenylhydrazin makes it imperative — when testing for mannose in presence of dextrose — to proceed in such manner that the risk of confusion through the presence of osazones shall be excluded. Even in cases where mannose is present, and where satisfactory crystals of mannose-hydrazone have been obtained on testing with phenylhydrazin compounds, it may happen constantly that the balls with sharp-pointed spines or the needles of dextrose-osazone will appear after a time when the precipitate and supernatant liquor have been left to stand for a few days. In such mixed precipitates characteristic crystals of mannose-hydrazone and of dextrose-osazone may be seen under the microscope lying side by side. Even on recrystallizing the mixed crystals from warm diluted alcohol, both kinds may reappear unless the recrystallization is carefully conducted. In his book* entitled "Physiology of the Carbohydrates," Pavy has published a number of microphotographs of osazone crystals as obtained from the hydrolysis of albumin and of a variety of other animal matters, — notably, muscular tissue, liver, spleen, and kidney, and of osazone crystals from blood and urine also. Many of these figures are exact representations of crystals obtained in this laboratory in numberless instances from the dextrose present in the products of the hydrolysis of the cellulose in woods of various kinds that were in process of being tested for mannan.

To avoid as much as may be possible the formation of dextrose-osazone, the mixture of the phenylhydrazin reagent and the solution under examination should neither be heated nor left to stand too long in the air. After allowing a reasonable time — about two hours — for the formation of the mannose-hydrazone precipitate, it is well to pour off the supernatant liquid and to remove the excess of the reagent by washing the precipitate with cold water. Since dextrose-osazone is soluble in glacial acetic acid, mannose-hydrazone may be freed from it either by drenching the "original precipitate" with this acid before examining the precipitate under the microscope, or, in case of need, a drop of the acid may be allowed to flow in upon the precipitate under the cover-glass on the slide of the microscope. This use of the glacial acid is of special importance in the case, which may happen not infrequently, where the spines on fringed or roughened globules of mannose-hydrazone are so small that the eye cannot determine whether they have sharp points, like needles, or blunt and bevelled ends. On mere inspection of such "sea-urchins," doubts would arise as to whether they were mannose-hydrazone or dextrose-osazone, though a recrystallization from alcohol will usually bring out crystals of mannose-hydrazone if any of this substance is really present. Any uncertainty of this sort may be removed by treating the globules at once with glacial acetic acid, which will dissolve the osazone balls and leave those of the hydrazone practically intact.

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* F. W. Pavy, Physiology of Carbohydrates, London, 1894. A German translation of this book, made by K. Grube, was published in Leipzig and Vienna in 1895.

Inasmuch as mannose-hydrazone is much less soluble in diluted alcohol than dextrose-osazone is, a large proportion of the latter will remain dissolved in the alcohol after the mannose-hydrazone has crystallized. Yet, again, it happens not infrequently that the dextrose-osazone, which forms in and upon a mannose-hydrazone precipitate when the latter has been allowed to stand for some time under its mother liquor, will be in the form of numerous minute, pale green, spiny balls, which are so much lighter and more flocculent than the mannose-hydrazone precipitate that they can be decanted off with the mother liquors, and also when the precipitate is washed with water. The facts that mannose-hydrazone may itself change to an osazone on being boiled with water, and that dextrose-hydrazone, when in presence of an excess of phenylhydrazin, is apt to be oxidized to dextrose-osazone, especially if the mixture be warmed, were pointed out some years ago by Fischer.

Behavior of Dextrose when Tested with Phenylhydrazin Acetate.

In the course of this research, the oxidation of dextrose-hydrazone to dextrose-osazone has been noticed very frequently when, in testing for mannose, the mixture of phenylhydrazin compounds and products of the hydrolysis of woods has been left to stand during several days. Conspicuous illustrations of it have been seen also in experiments where solutions of pure dextrose, as well as of "glucose" and "starch-sugar," were treated with phenylhydrazin compounds. For example, 1 cc. of a 0.2 per cent. solution of dextrose, treated with one drop of phenylhydrazin and two drops of glacial acetic acid and left to stand for several days, gave a small yellow amorphous precipitate in which were to be seen under the microscope occasional "sea-urchin" balls of dextrose-osazone with sharp spines. Similar results were obtained on repeating the test with a mixture of one drop of phenylhydrazin and six drops of glacial acetic acid. On removing the precipitate by filtration and adding some drops of the reagent to the clear filtrate, the yellow amorphous precipitate again appeared, and after a time crystalline balls ("sea-urchins") of dextrose-osazone were observed, although there was no special evidence that the yellow dust and the osazone crystals were in any way connected with one another. Similar results were obtained in other experiments where dilute solutions of dextrose were treated methodically with successive portions of phenylhydrazin compounds in the hope of discovering the nature of the yellowish amorphous precipitate, above mentioned, which forms when compounds of phenylhydrazin are added to dilute solutions of dextrose. Under the microscope this precipitate seems at first to be merely a fine amorphous dust from which no crystals have been obtained on treating with warm diluted alcohol and subsequently cooling and evaporating. But after the mixture has stood for several days, patches of a thin, greenish membrane, like the skin of a delicate fruit, are found admixed with the yellow dust, and after a while small crystalline masses of dextrose-osazone become visible, usually in the shape of little greenish balls thickly beset with fine, sharp-pointed spines, though sometimes bundles of needle-like crystals were noticed or bunches of needles radiating from a central point. The small, green, spiny balls are specially apt to form in or upon the green, skin-like patches. As was just now said, it does not appear that the formation of the spiny balls is dependent in any way on the presence of the yellow, amorphous precipitate; they come manifestly from the oxidation of the dextrose-hydrazone which has been present in the solution from the moment when phenylhydrazin was added to the dextrose. As contrasted with crystals of mannose-hydrazone, the crystals of dextrose-osazone formed under the conditions of this particular experiment are much more delicate. They consist usually of slender needles, often springing from a central point; even the spiny balls, above mentioned, seem to be really masses of needles closely impacted one against the others.

For these trials, solutions were prepared by dissolving either 0.2 grm. of pure dextrose (from Merck & Co.) in 10 or 5 cc. of distilled water, or 0.02 grm. of this dextrose in 10 cc. of water, and adding to 1 cc. portions of these solutions either 1, 2, 3 or 8 drops of solutions of phenylhydrazin compounds, prepared as described on page 14. After a few minutes the liquids thus treated became turbid, and after some hours the yellow precipitates just now described settled out. In numerous instances these yellow, amorphous, dust-like precipitates were removed by filtration soon after they had formed, and additional drops of the phenylhydrazin reagent were added to the clear filtrates. Sometimes this process of fractional precipitation and filtration was repeated again and again, but small quantities of the yellow precipitate formed anew as often as the trials were made, and after the lapse of some time crystalline balls of dextrose-osazone were almost sure to appear. In these trials, it seemed as if the phenylhydrazin acetate acted somewhat more quickly and more strongly to favor the oxidation of the dextrose-hydrazone and to induce the formation of the osazone crystals than the mixture of phenylhydrazin hydrochlorate and sodium acetate did.

It is noticeable that for the oxidation of dextrose-hydrazone time seems to be a more important factor than momentary heating of the materials upon the water-bath. Thus, when 1 cc. of a 1 per cent. solution of pure dextrose was boiled for half a minute with five drops of a dilute aqueous solution of phenylhydrazin, prepared by dissolving 1 cc. of the phenylhydrazin in 15 cc. of water, nothing but a small quantity of minute, dust-like particles separated; and a similar reaction occurred when 1 cc. of the 1 per cent. dextrose solution was boiled for half a minute with one drop of pure phenylhydrazin. On boiling half a cc. of a 10 per cent. dextrose solution with a drop of phenylhydrazin, some crystals of dextrose-osazone separated in the course of two or three days. In an instance where eight drops of a mixture of phenylhydrazin hydrochlorate and sodium acetate (prepared as on page 14) and 1 cc. of a solution of 0.2 grm. dextrose in 5 cc. of water were heated for one hour on a boiling water-bath, no crystals of dextrose-osazone were obtained, but only the usual yellow amorphous dust. These results are noticeably different from those obtained every day in the application of the common clinical test for dextrose, for the presence of common salt in this case would naturally elevate the temperature at which the liquid boils, and so accelerate the transformation of dextrose-hydrazone to dextrose-osazone.

Although the number and size of the mannose-hydrazone balls and crystals, which appear on the addition of the phenylhydrazin reagent to solutions of mannose, will often be seen to increase from hour to hour, or even from day to day, when the mixture is left to stand, it is still true in ordinary testing that the hydrazone balls and crystals appear much sooner than the osazone crystals, which would naturally be deposited later than the mannose-hydrazone, since they are not present in the solution at first but are formed there subsequently and somewhat slowly by way of oxidation. Hence, it happens that the speedy appearance, in any given case, even of imperfect or doubtful globules or crystals on the addition of the phenylhydrazin reagent, is of the nature of presumptive evidence that some mannose is present, and the experimenter should be encouraged to continue his search for it. Conversely, the total absence of balls or crystals until many hours after the application of the test would indicate that there is little chance of finding any mannose in that solution.

In comparison with the above mentioned trials with pure dextrose, others were made with commercial "glucose" and with "starch sugar." To one portion of a dilute solution of glucose there was added eight drops of the mixture of phenylhydrazin hydrochlorate and sodium acetate, and the mixture was heated on a boiling water-bath for an hour. The precipitate contained, beside the amorphous dust, many clusters of small, round globules that were opaque and thick, and it did not change in appearance in the course of three days. To another portion of the glucose solution sixteen drops of the phenylhydrazin reagent were added, the mixture was heated for an hour as before, and a similar precipitate was obtained that did not change in appearance on standing for two days. To yet another portion of the glucose solution twenty drops of the reagent were added and the mixture was heated as before. After several hours a few carpet-like patches of small, round, reddish globules were detected, but the appearance of the precipitate did not change in two days.

For the sake of comparison, the ordinary clinical test * for

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* As is well known, the formation of dextrose-osazone is an essential feature of a highly approved method of detecting sugar in diabetic urine. Descriptions of this test as employed by different chemists vary as to details, and some of the methods give better results than others. The method here described has given me excellent results. In the hands of Pavy, the ready formation and detection of osazones had led to a masterly exposition of the facility with which hexoses may be obtained by the hydrolysis of albuminoids and proteids.

dextrose was applied to several of the solutions used in the foregoing series of tests. Thus, 1 cc. of the dextrose solution (0.2 grm. in 5 cc. water) was added to a mixture of five drops of phenylhydrazin, ten drops of glacial acetic acid, and 1 cc. of a saturated solution of sodium chloride and boiled during two minutes. A heavy, canary-yellow precipitate fell, in which could be seen many pale green needles, like pine needles, as well as tufts of needles and masses of fan-like needles radiating from central points. On recrystallizing from warm diluted alcohol, still finer and more delicate needles were obtained; and on diluting the dextrose solution with twice its volume of water and again applying the clinical test, still finer crystals were to be seen. There could be no question as to their similarity with the crystals formed, as above stated, in mixtures of dextrose and phenylhydrazin compounds left to stand for some time at the ordinary temperature of the air.

These clinical tests were repeated in solutions made by mixing 1 cc. of pure dextrose solution with 2 cc. of healthy urine. The precipitates were seen to contain the same small, green, radiating needles and spiny balls of dextrose-osazone, as before, though the precipitate did not have the bright yellow color of that obtained in the absence of the urine. So, too, when mixtures of "glucose" and urine were treated. Many of the figures given by Pavy * well represent the osazone crystals obtained in these trials.

In several instances the clinical test for dextrose was applied also to products of the hydrolysis of the ivory nut, by adding phenylhydrazin, glacial acetic acid, and a solution of sodium chloride to a few drops of the liquor, and boiling for two minutes. The yellow precipitates obtained showed usually, under the microscope, mixtures of globules and rosettes of mannose-hydrazone commingled with the needles, sharp-pointed spiny balls, and brush-like radiations of dextrose-osazone.

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* Physiology of the Carbohydrates, London, 1894; notably the figure on page 51.

Remarks on other Globules which might be confounded with those of Mannose-hydrazone.

Beside the bare possibility that drops of free phenylhydrazin might contaminate a precipitate, as has been suggested by Pavy, and mentioned above on page 17, there are some other kinds of globules that need to be considered. In the products of the hydrolysis of birch wood, in particular, and in those from wood of the poplar, the willow, and the sugar-maple, also, though to a less degree, the precipitate produced by phenylhydrazin is found to contain numerous small, flat, pale globules, like minute drops of oil, of yellowish or reddish-yellow color. But from these globules no crystalline product has as yet been obtained. These globules have a very different appearance from the globules of mannose-hydrazone. As seen under the microscope they seem to be thin, lean, and flat, while the mannose-hydrazone disks appear to be thick and plump, and to be built upon a generous scale. Though somewhat soluble in glacial acetic acid, the oil-like globules are not so readily dissolved by this acid as are the spiny balls of dextrose-osazone. The fact that in the case of the maple wood oil-like globules appear simultaneously with those of mannose-hydrazone in the precipitate produced by the phenylhydrazin reagents, while in the case of birch wood an abundance of the oil-like drops are present, though no mannose-hydrazone could be detected, has made it imperative to try again and again to detect mannose in the products of hydrolysis from birch wood, though thus far without any success. Many samples of the birch wood have been hydrolyzed, both with hydrochloric acid and with sulphuric acid of varying degrees of concentration, but no mannose has been detected.

In one special trial, made on purpose to determine whether it was possible to avoid these globules, 22 grm. of powdered birch wood, from a tree felled on October 10, were leached thoroughly with ether, and with alcohol of 95 per cent., and were subsequently washed with water and partially dried before proceeding to boil the material for three hours in 220 cc. of hydrochloric acid of 5 per cent. After neutralizing the product of the hydrolysis with sodium hydroxide, it was tested with the phenylhydrazin reagents. Yellow precipitates, soon becoming red colored, were thrown down at once, both from the unevaporated and the evaporated liquor, and numbers of the small, oil-like globules were visible when the precipitates were examined with the microscope after standing for an hour. No indications of mannose-hydrazone appeared, though some pale green crystals of dextrose-osazone were seen after the mixture had stood for a day, and they increased in number on longer standing. Both the oil-like globules and the osazone crystals were soluble in glacial acetic acid.

Other kinds of globules, — dark and thick and often of reddish color, — of very different appearance from that of the oily drops just mentioned, have been encountered occasionally in the original precipitates produced on the addition of the phenylhydrazin reagents to products from the hydrolysis of woods. Usually these large, dark-colored globules, like most of the globules other than those of mannose-hydrazone which have been met with in this research, are soluble in cold glacial acetic acid when recently prepared, though they become much less soluble in the acid after long standing. Occasionally they are somewhat difficultly soluble in the acetic acid even when fresh; indeed, some specimens obtained on testing commercial "glucose," as mentioned above on page 26, and in one instance on testing pure dextrose, were hardly any more soluble in glacial acetic acid than mannose-hydrazone itself, and their appearance was remarkably like that of mannose-hydrazone globules, though no crystals could be obtained from them by treatment with diluted alcohol. According to Tollens,* Hirschl, on meeting with analogous "braune Kügelchen" when testing urine for sugar, suggested that they might perhaps depend on the presence of gluconuric acid, — an idea which was naturally more probable in the case of the urine he was testing than it would be for dextrose or "glucose," for urine not infrequently contains gluconuric acid.

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* Handbuch der Kohlenhydrate, **2.** 100.

Details of the Method of applying the Phenylhydrazin Test for Mannose.

In the actual testing of woods for mannan, a convenient method of procedure is to boil about 20 grm. of the finely powdered wood, regarded as dried at 100° C., in 200 cc. of hydrochloric acid of 5 per cent. during three hours, over a free flame, to neutralize the filtered product of the hydrolysis with dilute caustic soda (1 : 8) and to test 1 cc. portions of the unevaporated filtrate by adding twelve drops of the phenylhydrazin reagent. In case much mannose is present, it is a matter of indifference whether the reagent used has been prepared by mixing phenylhydrazin and acetic acid, as has been explained above on page 14, or by mixing phenylhydrazin hydrochlorate and sodium acetate; but when any uncertainty exists as to the quantity of mannose to be expected, it is well to make two separate tests, — one with each of the reagents. On the whole, the mixture of phenylhydrazin and acetic acid gives rather better results than the hydrochlorate and sodium acetate, though the latter sometimes yields cleaner and clearer crystals of mannose-hydrazone, since the confusing, spiny crystals of dextrose-osazone are apt to form somewhat sooner in the acetic solution than in the other. Experience has taught that, when operating on the unevaporated liquor, it is best to add as much as twelve drops of the reagent rather than a smaller number. After the addition of the reagent the mixture is left to stand at rest for a couple of hours, and is then examined with the microscope. If the result of this examination leaves nothing to be desired, the experiment is regarded as finished; but if only a small quantity of mannose-hydrazone is visible, the mixture should be left to stand over night.

If the precipitate obtained on thus testing the unevaporated product of the hydrolysis is seen to contain actual crystals of mannose-hydrazone when examined under the microscope, no further steps need be taken. But if only balls appear, whether smooth or roughened, the supernatant liquor is poured off from the precipitate and the latter is washed with cold water, by way of decantation, to remove common salt and other extraneous matters, as has been explained on page 17, and the precipitate is dissolved in warm, somewhat diluted alcohol (3 : 1). On allowing the alcoholic solution to crystallize, with proper precautions, the characteristic crystals of mannose-hydrazone will appear. If, through too rapid evaporation of the alcohol or from any cause, no thoroughly satisfactory crystals appear on the first trial, the process of recrystallization from alcohol should be repeated until all doubts are removed. It is worthy of remark that better results have been obtained, as a general rule, when the alcoholic solution has not been filtered, but has been simply decanted from or even left to stand in the tube in which the solution was made.

In case neither crystals of mannose-hydrazone nor the characteristic balls of this substance appear when the unevaporated product of the hydrolysis has been tested several times in separate portions of the liquor, each of 1 cc. or more, the remainder of the liquor is evaporated to dryness on a water-bath, the residue is taken up with a small definite quantity of water, usually 10 cc., and 1 cc. portions of this solution are tested with the phenylhydrazin reagents, very much as before, though in this case sixteen drops of the reagent may be added instead of the twelve drops that are used with the weaker, unevaporated liquor. In case no satisfactory precipitate is obtained on testing 1 cc. portions, then larger quantities are taken: sometimes 3 cc., and sometimes the whole of the residual liquor is treated at once with the reagent.

One advantage gained by working methodically with similar weights of the woods and definite volumes of the solutions is that rough indications may be obtained as to the relative quantities of mannan contained in the different woods. Manifestly, a heavy precipitate of balls and crystals of mannose-hydrazone obtained from 1 cc. of the unevaporated liquor will show that the wood under examination contains an abundance of mannan, while a meagre precipitate obtained from several cc. of an unevaporated liquor indicates that there is less mannan in this wood than there was in the other. Similar relative indications are to be had when the product of the hydrolysis is evaporated, and 1 or 3 or any larger number of the 10 cc. of liquid thus obtained are tested. It is easy to demonstrate in this way that while pine wood contains much mannan, comparatively speaking, the wood of the sugar-maple contains less of it, and less at some seasons than at others, while in the wood of the gray birch, taken in the quantity and under the conditions above mentioned, I have been unable as yet to detect any mannan at all. According to this rough method of estimating quantities, it appears: —

1. That an abundance of mannan is contained not only in date stones and the ivory nut,

   but also in the flesh of a ripe cocoa nut (*Cocos nucifera*) bought in the Boston market on May 8;*

   and in the trunk-wood of white pine trees (*P. strobus*), 2.5 and 3 inches in diameter, felled respectively on December 14 and in late August, at Hingham, Mass.;

   in the trunk-wood of a pitch pine tree (*P. rigida*), 2.5 inches in diameter, felled December 27 at the Bussey Institution;

   in the trunk-wood of a Norway spruce (*Picea excelsa*), felled early in November at the Bussey Institution, — a piece of the trunk-wood 2.5 inches in diameter was taken from near the top of a tall tree;

   in the wood of a limb of the Japanese larch (*Larix leptolepis*), 0.5 to 0.75 inches in diameter, cut off near the ground from a tall tree on January 23 at the Bussey Institution;

   in the wood of a limb of the hemlock spruce (*Tsuga Canadensis*), 2 inches in diameter, cut off on February 1 at Hingham, Mass.;

   in the wood of a red cedar (*Juniperus Virginiana*), 2.5 inches in diameter, felled December 27 at the Bussey Institution;

   in the wood of a limb of the white cedar (*Chamæcyparis sphæroidea* [The *Cupressus thyoides* of Linnæus]), 2 inches in diameter, cut off on January 25 at Hingham, Mass.;

   and in the seeds of white clover† (*Trifolium repens*).

   Thoroughly characteristic evidence of the presence of mannose was seen on examining the precipitates of mannose-hydrazone obtained from 1 cc. of the unevaporated, neutralized product of the hydrolysis of each of the foregoing materials.

2. That strong indications of mannose — though the quantity of it is manifestly less than that exhibited by the substances mentioned in the foregoing list — have been detected in the unevaporated product of the hydrolysis

   of the wood of a limb, 2.25 inches in diameter, of the sugar-maple (*Acer saccharinum*) cut off January 21 at Hingham, Mass.;

   of the seeds of red clover (*Trifolium pratense*);

   and of orange peel.‡

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* Compare E. Schulze, as cited in Tollen's Handbuch der Kohlenhydrate, 1895, **2.** 228.

† Muntz and Bourquelot, and Hérissey also (Comptes Rendus, 1900, **130.** pp. 340, 731, 1719), have detected mannan in clover seeds of several different species.

‡ Flataŭ and Labbé (Bulletin de la Société Chimique, 1898 (3.), **19.** 408, as cited in Chemisches Centralblatt, 1898(1), 1238) have obtained mannose directly by macerating fresh orange peel with alcohol or with water.

3. That good evidence of the presence of mannose has been seen on testing the *evaporated* product of the hydrolysis

- of the wood of the limb of the sugar-maple, 2.25 inches in diameter, cut off on January 21;
- of the outer and to a less extent of the inner trunk-wood of a sugar-maple tree, 6 inches in diameter, felled on October 17 at West Newfield, Me.;
- of the seeds of the white pine;
- of the seeds of the Norway spruce;
- of the seeds of the horse chestnut (*Aesculus hippocastaneum*);
- of the small twigs on the limb of the Japanese larch, above mentioned;
- of the seeds of the red clover;
- of orange peel;
- of the root of a Jerusalem artichoke (*Helianthus tuberosus*);
- of two samples of the roots of chicory plants (*Cicorium intybus*) dug up at the Bussey Institution, one in late September and the other on May 6, before flower stalks had begun to develop;
- of dandelion roots (*Taraxacum officinale*) dug up at the Bussey Institution on May 6, when the plants were on the point of blossoming;
- of the roots of asparagus plants (*Asparagus officinalis*) dug up on October 3, at Hingham, Mass.

No mannose was detected in the unevaporated products of the hydrolysis of either of the substances in this list, with the exception of those from the maple wood felled in January, the red clover seed, and the orange peel, and it was noticed that much stronger reactions for mannose were obtained from the evaporated products of the hydrolyses of these three substances than had been got previously on testing the unevaporated liquors.

The great mass of soft vegetable matter and of water contained in the Jerusalem artichoke made it necessary to proceed with special care in testing for mannose in the products of the hydrolysis. In one case where 20 grm. of the natural, undried root, taken from the ground in late November, were hydrolyzed with 200 cc. of 5 per cent. hydrochloric acid, excellent crystals of mannose-hydrazone were obtained on testing 1 cc. portions of the evaporated liquor. But no satisfactory result was got in a subsequent trial where 20 grm. of the crushed root were left in contact with an equal quantity of concentrated sulphuric acid during thirty-six hours, and the mixture was boiled for three hours after the acid has been diluted with water to a strength of about 5 per cent. (1.033 sp. gr.). After neutralizing with dilute caustic soda, evaporating to dryness and taking up with 10 cc. of water, no mannose-hydrazone was detected. But on drying a quantity of the root and treating 20 grm. of the dry powder with sulphuric acid in the same way that the natural root had been treated in the foregoing experiment, there was no difficulty in obtaining crystals of mannose-hydrazone on testing the liquor after it had been neutralized with marble dust and evaporated to dryness.

4. That traces of mannose were detected on testing the solutions taken up from the evaporated product of the outer trunk-wood of sugar maples, 5 and

4.5 inches in diameter, felled respectively on May 1 and on May 30 at West Newfield, Me., and also that doubtful traces were noticed in the wood of a root 1 to 1.5 inches in diameter of a sugar-maple dug up in November at West Newfield, though no indications of mannose were seen on testing the unevaporated liquors.

5. That no mannose was detected in the unevaporated product of the hydrolysis

of small white beans (*Phaseolus*); or

of the small terminal twigs of a gray birch (*Betula populifolia*), collected November 2 at the Bussey Institution, from a tree 3 or 4 inches in diameter and some 15 feet high.

In neither of these instances was the evaporated liquor tested.

6. That no traces of mannose could be discovered either in the evaporated or the unevaporated liquors from the hydrolysis

of the inner trunk-wood of the sugar-maple, felled on May 30;

of the trunk-wood of several gray birches (*Betula populifolia*), felled at different times and seasons, but especially in the autumn and winter;

of the seeds of the gray birch;

of the wood of a limb, 4.5 inches in diameter, cut off from a willow tree (*Salix alba*) on January 10, at the Bussey Institution;

of the trunk-wood of a poplar tree (*P. tremuliformis*), 4 inches in diameter, felled December 14 at Hingham, Mass.;

of the trunk-wood of the so-called leather wood [or moose wood] (*Dirca palustris*) one inch in diameter, procured on April 11 at West Newfield, Me., — no starch grains could be detected with the microscope in this wood;

of orange seeds;

of peas;

of the root of the yellow pond lily (Beaver root) (*Nuphar advena*).

Since an abundance of crystalline osazone balls ("sea-urchins") were obtained from the product of the hydrolysis of the lily root, it is evident that there is contained in it some matter (presumably cellulose) capable of yielding dextrose or some other kind of sugar.

The points of chief interest in the foregoing enumeration of tests are: —

1. The presence of mannan in the trunk-wood of sugar maple trees, especially in the wood of trees felled during the period of hibernation, — that is, in January and October, — and the comparatively small quantities detected in maple trees felled at the beginning and the end of May, — that is, during or after the formation of new suits of leaves upon the trees. I have insisted in another place* on the improbability of the popular opinion that

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* Bulletin of the Bussey Institution, 1900, **2.** 437; also 1897, **2.** 386 *et seq.*

Mannose from Oxidation of Mannite

3.24
46
3.70

15
13
45
15
195

21 in
24
80 in
32
34 the

there is starch enough stored up in maple trees in the autumn to account for all the sugar and the physiological work that is exhibited by these trees in the spring. But the results here recorded make it evident that mannan, as well as some starch, is stored as reserve food in the wood of the sugar maple. It may be remarked yet again that the old idea that large quantities of starch are stored in the wood of trees in the winter, arose from the employment of improper methods of estimating starch by chemists at the middle of the last century.

2. The apparent absence of mannan from the wood of the gray birch, the poplar, and the willow. Of course, the negative results obtained in these cases cannot be regarded as proof positive of the entire absence of mannan. From the mere fact that they are negative, these results can carry no such conviction of certainty as do the positive results obtained in the case of the sugar maple, for example, for these last were simply unmistakable and admitted of no shadow of doubt or uncertainty; but it is safe to say that the observed absence of mannan from the wood of the trees now in question shows clearly that some of the chemico-physiologic processes, which occur in the wood of these trees, must be of different character from those that occur in the maple tree and in coniferous trees.

3. The detection of mannan in orange peel, but not in orange seeds.

4. The occurrence of small quantities of mannan in the tuberous roots of the Jerusalem artichoke, as an adjunct to inuline.

5. The detection of mannan in horse-chestnut seeds, as an adjunct to starch.

6. The presence of mannan in the storage roots of chicory, dandelion, and asparagus; and

7th, and perhaps most important of all, the presence of but a small quantity of mannan in the seeds of the white pine and the Norway spruce, although the wood of these trees and of other pine trees contains an abundance of mannan, as had been shown already by the experiments of several other chemists beside myself. The poverty of the pine seeds was brought out very clearly on attempting to estimate quantitatively the mannan in the wood of pine trees felled at different seasons of the year, as well as that in the seeds of this pine. To this end, samples of finely powdered material were prepared from the wood of a pine tree three inches in diameter that was felled in Hingham, Mass., in the last week of August, 1900; from the wood of a tree 2.5 inches in diameter felled in Hingham on December 14, 1901, and from white pine seeds bought of a Boston seedsman. Weighed quantities (25 grm. in each case) of the powdered woods were leached with four successive portions of cold water, then partially dried and washed with cold alcohol of 95 per cent. Subsequently, the samples were soaked for two hours in a cold 2 per cent. solution of caustic soda, and the soda was washed out with water with the aid of the filter pump. Each of the washed samples was boiled over a free flame during 4.5 hours in 250 cc. of hydrochloric acid of 5 per cent.; the filtrate was neutralized with dilute soda lye (1 : 8), and to the clear filtrate there was added 20 cc. of a mixture of one part phenylhydrazin and two parts glacial acetic acid. After being allowed to stand for a couple of hours, the precipitated mannose-hydrazone was collected on a tared filter; to the yellow filtrate more of the phenylhydrazin acetate was added, and the mixture was allowed to stand over night. A new precipitate of mannose-hydrazone formed, and it was collected on a new filter. Meanwhile, the residual wood left by the first hydrolysis was rinsed with water and treated again with 250 cc. of hydrochloric acid of 5 per cent. and boiled during 4.5 hours. The liquor was then neutralized and treated as before. No precipitate of mannose-hydrazone formed in the course of an hour and a half, but after the mixture had stood over night a quantity of mannose-hydrazone was in evidence. This precipitate was collected on a new filter, a new portion of the reagent was added to the filtrate, and the small new precipitate that formed was collected in its turn. It was somewhat contaminated with crystals of dextrose-osazone. All these precipitates were washed with cold, distilled water, and subsequently with cold alcohol of 95 per cent. to remove the osazone; finally, they were dried at 100° C. and weighed. The residual wood was hydrolyzed for a third time by boiling it with 250 cc. of 5 per cent. hydrochloric acid during 4.5 hours, but no precipitate of mannose-hydrazone was obtained on testing the neutralized liquor. It hardly needs to be said that the method of estimating mannan above given is far enough from being perfect. It is not probable that all the mannan in the wood was hydrolyzed in these experiments, or that it is ever in our power to convert the mannan in any sample of wood completely into mannose, for some or even much of it will be apt to remain combined with cellulose so firmly as to resist inversion, while a part of it will probably be changed to "wood dextrin" by the action of the hydrolyzing acid in the same way that cellulose is thus changed. Moreover, the precipitation of the mannose-hydrazone must necessarily be incomplete because this substance is by no means totally insoluble in water, and because of the risk of the precipitate's becoming unduly contaminated with dextrose-osazone if it is left to stand too long in order that it may accumulate and increase. But it is to be observed that the results here recorded are comparable one with the other, since the trials were made under similar conditions, though it would probably have been better, as I can now see, to have evaporated the products of the hydrolyses before adding the reagent.

For the analysis of the pine seeds 27 grm. were taken and treated in a slightly different way from the foregoing, in that the material was not subjected to the preliminary leachings with water, alcohol, and dilute soda lye which had been applied to the pine wood. The powdered seeds were simply boiled at once with the hydrochloric acid of 5 per cent., and the hydrolysis was repeated thrice as before. No precipitate of mannose-hydrazone was obtained on testing the neutralized product of the third hydrolysis, and since the liquors from the first and second hydrolyses manifestly contained but little if any of it, they were evaporated on a water-bath to rather less than half their original bulk. After filtering off a certain amount of gelatinous matter that formed on neutralizing the acid liquors, the two solutions were treated, together, with the phenylhydrazin acetate, but no precipitate of mannose-hydrazone could be detected.

In brief, it appeared as the result of the quantitative experiments, made in the manner and under the conditions above described, that

3.61 per cent. of mannose was obtained from the wood (regarded as dried at 100° C.) of the pine tree felled in August, and

1.86 per cent. of mannose from the wood of the pine tree felled in December, while no mannose was got from the pine seeds.

The experiment with the pine seeds was repeated at once qualitatively and the result above stated was practically verified, for only small traces of mannose could be detected, and that after much trouble. The qualitative test was more severe than the other, in that the product of the hydrolysis was evaporated to dryness, whereas in the quantitative test the partially evaporated liquor was treated. In this qualitative experiment 18 grm. of the powdered seeds were hydrolyzed, the neutralized product of the hydrolysis was evaporated to dryness, and the residue taken up with 20 cc. of water. When as much as 3 cc. of this solution were tested some crystals of mannose-hydrazone were finally obtained, after much waiting and, so to say, coercion; but when quantities no larger than 1 cc. of the liquid were examined the tests were less satisfactory, and no indications at all of the presence of mannose-hydrazone were got when the unevaporated, neutralized product of the hydrolysis was tested.

The examination of the seeds of the Norway spruce, as previously mentioned, was made for the express purpose of confirming or refuting the results of the tests made with the seeds of the white pine. As will appear from the following statement, the results of the trials with the spruce seeds were almost absolutely similar to those got with the pine seeds. Twenty-five grm. of the spruce seeds were boiled for three hours with 220 cc. of hydrochloric acid of 5 per cent., the acid was neutralized with sodium hydroxide, and tested with the phenylhydrazin reagents. No evidence of mannose was detected in the unevaporated liquor, while a few mannose-hydrazone crystals from one portion of the evaporated liquor and none at all in trials made upon two other portions of the liquor. Manifestly, there was but little mannose obtainable from these seeds.

With regard to the quantity of mannan in the trunk-wood, it would of course be wholly improper to argue from the results of a couple of experiments that there is usually more mannan in the pine tree in August than in December; but the results above given point to the necessity of studying more carefully than has been done hitherto the general question as to the times and seasons when the pine and other evergreen trees store up their reserve food.

Probably there are other Reserve Foods in Trees beside Starch and Mannan.

The question what kinds of reserve foods are stored in trees that contain little starch and no mannan is still an open one. As has appeared from experiments described on pages 391, 443, 448, and 465 of Volume II of the Bussey Bulletin, I have been unable to detect much if any galactan in the wood of trees or enough starch to account for the abundant growth of leaves, etc., in the spring. The practical absence of xylan (wood gum) from the wood of coniferous trees in which mannan abounds and the presence of much xylan in the wood of trees that carry no mannan, naturally suggest the thought that xylan might perhaps be an important kind of reserve food for the trees in which it occurs. But there are a number of considerations that weigh against this idea, — notably, the fact that xylan is not a fit food for animals, while mannan and starch are both excellent, and that experiments have not shown that xylan supplies food to the young plant at the moment of germination, as starch and inuline, mannan and galactan do. The comparative indestructibility of xylan when wood or the like is left to decay in the soil, serves to emphasize the non-physiological attributes of this substance. It appears, indeed, that xylan is probably less important than cellulose as a means of feeding plants, — that is, as affording a supply of reserve food.

In respect to many seeds, — such as pine seeds, for example, — it is known that fatty and oily matters play a highly important part for feeding the young plant at the moment of germination. But as regards the actual wood of trees, I cannot but feel for my own part that it will be discovered some day that there is often contained in it another "hemicellulose," comparable to starch and mannan (and to inulin and galactan also), which serves as these substances do as reserve food for the production of leaves and new wood in the spring.

It is noticeable that the finding of mannan in the wood of sugar-maple trees — which yield great quantities of sucrose at the moment when vegetation is renewed in the spring — goes to show that there must be a peculiarly intimate connection between these two substances. It may well be argued that the easy conversion of mannan to sucrose is a fact of general import common to a great variety of plants. There are, indeed, numerous detached observations, scattered here and there in chemical literature, which point to this conclusion. As an example illustrating it may be cited the observation of Rongger [*] who found cane sugar, among other things, in the seeds of *Picea excelsa* and *Pinus cembra*. Numerous observations have shown that mannose is a constant constituent of the wood of coniferous trees, while, as has just been stated, I have been able to detect no more than traces of it in the seeds of *Pinus strobus* and *Picea excelsa*.

There can, moreover, be little doubt that the mannite so frequently found in various plants is a concomitant of mannan that has been stored up in those plants. In support of this view may be mentioned the well-known fact that in many instances it is only after the vegetable matters have undergone "fermentation" that mannite has been detected in them.

What kinds of Woods are capable of Nourishing Animals?

In the light of the knowledge that the wood of coniferous trees may at certain times and seasons contain considerable quantities of mannan, which is an approved food for ruminating animals, there would seem to be need of studying anew an old and somewhat disputed, not to say despised question, as to whether or not sawdust may have any value as cattle food when used instead of straw as an ingredient of rations for maintaining idle animals. Reports have several times been made, some years since, by German farmers who claimed to have obtained useful, practical results on feeding sawdust to neat cattle; while Professor F. Lehmann's [†] digestion experiments with sheep went to show that sawdust is practically indigestible and useless as food for these animals. It would be interesting to know what kinds of woods were administered to the animals in all these cases and at what times in the year the trees had been cut.

Kellner [‡] found that oxen fed, per head and dry, with 2 kilo. of fine, spruce sawdust mixed with 0.4 kilo. of molasses and 7 kilo. of cut hay ate the fodder freely, and digested 9.4 per cent. of the carbohydrates of the sawdust and 8.4 per cent. of its cellulose, or, together, 17.7 per cent., — against 12.9 per cent. of the carbohydrates and 23.1 per cent. of the cellulose (together 36 per cent.) in the straw of winter grain, when such straw was fed to the animals instead of the sawdust. He argues that, in times of dearth, fine, fresh spruce sawdust may well be used to replace straw in the rations of idle oxen, and that, when properly balanced by appropriate nitrogenous food, it may be regarded as possessing about one half the nutritive value of straw for maintaining oxen.

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[*] Die landwirthschaftliche Versuchsstationen, 1898, **51.** pp. 89, 93, 94. See also E. Schulze, ibid., 1901, **55.** 267.

[†] Landwirthschaftliche Jahrbücher, 1895, **24.** page 115 of Ergänzungsband I. Favorable practical results by O. Lehmann are cited in Hoffmann's Jahresbericht, 1893, **36.** 340.

[‡] Abstract in Biedermann's Centralblatt für Agriculturchemie, 1895, **24.** 164.

The Lead Acetate Test for Mannose.

Beside phenylhydrazin, there is another test for mannose that is excellent when applied to not too dilute solutions of the pure substance, but is of only limited applicability because of the liability that it will be interfered with or even vitiated by the presence of substances other than mannose in the matters to be tested. Reiss* has insisted strongly on the fact that, among all the sugars, mannose ("seminose") alone yields a precipitate when a neutral aqueous solution of it is mixed with a solution of basic acetate of lead. Although this mannose-lead precipitate has a decidedly characteristic appearance, and in spite of the fact that it is mentioned as a test in the standard text-books,† comparatively little attention seems to have been paid to it by chemists. I was surprised at this silence when my attention was first directed to the subject, for it is evident that the test is an excellent one when applied under fit conditions; but on seeking to apply it more freely I soon discovered a very serious difficulty in that almost all samples of the sugars obtainable in commerce, even those reputed to be chemically pure, contain traces of impurities which in presence of the basic lead acetate may cause cloudiness, faint precipitation, or sometimes a precipitate so distinctly appreciable as to preclude the possibility of testing for mannose in such solutions unless special precautions be taken. It appears from my own experience that, while no precipitate is produced when ordinary normal lead acetate is added either to solutions of sucrose or dextrose, both these sugars, as ordinarily prepared, may yield precipitates with basic lead acetate. In repeated trials, slight precipitates fell when a drop of the basic lead acetate * was added to solutions of so-called chemically pure sucrose, dextrose or maltose; and although these precipitates were of different appearance from the mannose-lead precipitate, they were still sufficiently like it to prevent the application of the test when any approximation to delicacy was required. These alien precipitates, though of similar color (white) to the mannose-lead precipitate, do not exhibit the gelatinous appearance of the latter. They are much more pulverulent and soon settle to the bottom of the test-tube. It was easily shown, by the method of fractional precipitation, that these pulverulent precipitates were caused by impurities in the sugar. On adding a solution of the basic lead acetate, a drop at a time and at intervals, to a solution of sucrose or of dextrose and filtering off the precipitates produced by each drop, it was seen that precipitation soon ceased and that the formation of the precipitates had nothing to do with the actual sugar. Usually only a slight milkiness was produced even by the second drop of basic lead acetate added to the filtrate from the precipitate caused by the first drop. By proceeding in this way with mannose solutions that were known to contain traces of calcium

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* Landwirthschaftliche Jahrbücher, 1889, **18.** 753, 754. Cf. E. Fischer and Hirschberger, Berichte der deutschen chemischen Gesellschaft, 1889, **22.** 1155.

† For example, in Lippmann's Chemie der Zuckerarten, page 335; and Tollen's Handbuch der Kohlenhydrate, **2.** 115.

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* Several different preparations of basic lead acetate were used in these experiments, and it is noteworthy that while all of them did good service, some did better than others. Two samples, prepared by heating during five or ten minutes an excess of litharge in a solution of normal lead acetate,* gave good results. Perhaps the method of heating for ten minutes may be better than heating for half an hour as recommended by the books. For most of my trials the reagent was prepared after Parrish † by boiling together for half an hour, in a flask with a reflux condenser, a mixture of 6.2 grm. of normal lead acetate, 3.6 grm. litharge, and 24 cc. of water. The product was filtered and kept in well-stoppered bottles. A sample of basic lead acetate, prepared according to Wiley,‡ did not give so good a precipitate with mannose as did the Parrish preparation or those prepared roughly as above described.

* Compare W. T. Brande's Chemistry, Philadelphia, 1863, page 598.

† E. Parrish, Introduction to Practical Pharmacy, Philadelphia, 1859, page 538.

‡ H. W. Wiley, Principles and Practice of Agricultural Analysis, Easton, Pa., **3.** 101.

sulphate, the second and third precipitates exhibited the characteristic gelatinous appearance of the mannose-lead compound even more emphatically than the first precipitate did. But since the mannose-lead precipitate is readily soluble in an excess of the basic lead acetate, no little care must be exercised when testing for mannose in this way. It is easy, however, thus to detect mannose when any considerable quantity of it is present, as in the product of the hydrolysis by dilute sulphuric acid of date stones, the ivory nut or pine wood. After removing all but the last traces of the acid by neutralizing with lime- or barium-carbonate, evaporating to dryness, taking up with warm, strong alcohol and applying the lead test after the alcohol has been driven off, a satisfactory reaction may be obtained.

Many attempts were made to apply the test with basic lead acetate to the product of the hydrolysis with 5 per cent. hydrochloric acid of substances containing mannan, after having removed chlorine by means of moist silver acetate or by a solution of this acetate in hot water. In solutions that contain a sufficient quantity of mannose, the characteristic lead compound may readily be precipitated by proceeding in this way, but in dilute solutions the reaction is apt to be unsatisfactory; and since the solution always holds dissolved a little silver acetate, — because this substance is slightly soluble and because an excess of it is used, — discolorations will occur through the decomposition of the silver salt when any attempt is made to concentrate the liquid by evaporation upon a water-bath. It is not difficult, however, to remove the silver from the mannose solution by means of clean chips of metallic lead, and the fact that on adding a fresh bit of lead it remains untarnished shows that the whole of the silver has been thrown down. Here again the mannose solution will react freely with basic lead acetate, provided it is strong enough, though the reaction is feeble if the solution is dilute. It is true that in this case the solution can be concentrated upon the water-bath without trouble before applying the lead acetate; though, after all has been said and done, the liability of vegetable matters to contain ash ingredients, notably sulphates, tends in many cases to cast a doubt on the lead-mannose reaction unless there is enough mannose present to visibly override the reactions that may be caused by traces of impurities. It has been noticed, moreover, in some instances, that by the reaction of silver acetate on the metallic lead employed to remove the excess of silver from the solution enough lead acetate may be formed to prevent the separation of the mannose-lead precipitate which is known to be soluble in an excess of this reagent. It was found, indeed, on trial that a small sample of the gelatinous mannose-lead precipitate gradually dissolved on being treated with a solution of normal lead acetate; and that, when a small quantity of a solution of sodium acetate was mixed with a weak solution of mannose, no precipitate formed therein on the addition of a drop of basic lead acetate, although in the absence of the sodium acetate a precipitate fell readily. In addition to these difficulties, it is to be remembered that solutions of gums of various kinds, as well as of pectin and vegetable mucilage, are readily precipitated by basic lead acetate, even from highly dilute solutions. According to Staedeler* dextrin also may yield a gelatinous precipitate when a concentrated solution of it is mixed with basic lead acetate.

It needs to be said, however, that instances may occur where, although the lead acetate would be of no use as a distinguishing test for mannose, it might serve fairly well for separating this sugar from an aqueous solution that contained it naturally beside various other soluble vegetable matters. In this event, the mannose might be recovered from the solution in a somewhat more concentrated form than it was before by precipitating it, together with gums, etc., by means of basic lead acetate, washing the precipitate with cold water, and decomposing it either with sulphuretted hydrogen — in presence of acetic acid — or with dilute sulphuric or hydrochloric acid, or by means of carbonic acid gas. In experiments made to test this method of procedure, a small part of the mannose-lead was put aside, while a slight excess of dilute sulphuric or hydrochloric acid was added to the remainder. After time enough had been allowed for the decomposing action of the acid, the reserved portion of the precipitate was stirred in to neutralize the excess of acid. The mannose solution was separated by filtration, evaporated to a small bulk, taken up with water and again filtered, if need were, before testing with phenylhydrazin. Or, if dilute sulphuric acid is used, a slight excess of it may be added to the whole of the precipitate, and the mixture may be drenched with strong alcohol after the acid has had time to act. After filtering, the alcohol is expelled, the excess of acid is neutralized, and the phenylhydrazin test applied. When carbonic acid gas was used for decomposing the mannose-lead precipitate particularly clear solutions were obtained. In this case, the filtrate from the lead carbonate was evaporated to a thick syrup which was treated with alcohol, somewhat diluted (3 : 1), and again filtered. After expelling the alcohol, the aqueous solution was tested with phenylhydrazin. Trials with oxalic acid as the agent for decomposing the mannose-lead precipitate went to show that, while a cold solution of one part oxalic acid in ten parts of water gave good results, the use of this acid was, on the whole, rather less satisfactory than that of either of the other acids above mentioned.

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* Annalen der Chemie und Pharmacie, 1859, **111.** 26 *note.*

In conducting this research I have been most ably seconded by my assistant, Mr. F. T. Dillingham. To him my best thanks are due. I am indebted also to my former assistant, Mr. H. W. Haynes, who labored with me assiduously in tentative efforts which opened the way to an easier and more accurate method of distinguishing mannose-hydrazone than was at that time known to us.

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For further information, apply to

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HARVARD UNIVERSITY

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No. 3. — *Notes on the Occurrence of Mannan in the Wood of some kinds of Trees, and in various Roots and Fruits.*

By F. H. Storer, Professor of Agricultural Chemistry.

The presence of mannan in the wood of certain coniferous trees has been detected by several chemists, and the generality of its occurrence in such trees has been confirmed and illustrated by numerous experiments made in the Bussey laboratory.

As has been explained in a previous article,* some evidence was met with in the course of those researches which pointed towards the conclusion that mannan may perhaps be more abundant in pine trees at certain seasons of the year than at others, and it was for the purpose of testing this supposition that a series of new experiments has been carried out on a somewhat different plan from the earlier tests.

Once a month during eleven months of a whole year a limb from 1.5 to 2 inches in diameter was cut from a young and thrifty white pine tree (*P. strobus*)† and reduced to splinters which were dried and ground to powder. A quantity of the powder equal to about twenty grams of matter reckoned as dry at 100° C. was boiled in a flask, with reflux condenser, during three hours with ten times as many cc. of hydrochloric acid of 5 per cent. as there had been taken grams of the air-dried material. For example, 220 cc. of the acid would be used upon 22 grams of the powder. At the end of the hydrolysis the contents of the flask were left to stand during several hours before filtering, the undissolved matter was washed with 20 cc. of water, and the washings added to the filtrate. Ten cc. of the well-mixed liquid were taken up and neutralized with extreme care with a solution of sodium hydroxide (1 : 8), using phenolphthalein as an indicator whenever possible, and taking pains to leave the liquid very slightly acid rather than alkaline. The liquid thus neutralized, or nearly neutralized, was divided into fractions and tested at once with a solution of phenylhydrazin acetate prepared by shaking together 1 cc. of phenylhydrazin, 2 cc. of glacial acetic acid, and 10 cc. of water.

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* Entitled "Testing for Mannose." In Bulletin of the Bussey Institution, 1902, **3.** 13.

† I am greatly indebted to my colleague, Mr. Edmund Hersey, for the trouble he has taken to collect, from a small plantation on his estate at Hingham, samples of limbs from pine trees which should be as nearly as possible comparable one with the others, though taken at different seasons and from different trees. Many other samples of woods and other materials were procured for me by Mr. Hersey and by other friends.

For the sake of gaining an approximate idea in each instance of the quantity of mannose in the product of the hydrolysis, the phenylhydrazin reagent was applied methodically as follows: The neutralized liquor was divided into several fractions each of 1 cc. The first of these fractions remained undiluted, but to the second fraction 1 cc. of water was added, to the third fraction 2 cc. of water, to the fourth 3 cc., and so on, the series of dilutions being carried as far as was thought to be by any possibility necessary. To each of these fractions 12 drops of the aforesaid phenylhydrazin acetate reagent were added, the mixtures were left to stand during at least two hours, and the precipitates were examined under the microscope to see if they contained balls or crystals of mannose-hydrazone. In case none of this substance appeared at first, the precipitates were examined at intervals during forty-eight hours, and it was only after this long-continued scrutiny that the absence of mannose-hydrazone was admitted. By this method of testing — which was applied not only to the examination of pine wood but to all the other materials described in this article — it is easy to assure one's self that some things contain much more mannan than do others. Thus mannose-hydrazone was detected in ten successive fractions of the unevaporated product of the hydrolysis of 10 grm. of clean buttons cut from the ivory nut (*Phytelephas macrocarpa*), — that is to say, mannose-hydrazone was found not only in the first undiluted fraction but in the nine following fractions in which 1 cc. portions of the product of the hydrolysis had been diluted respectively with 1, 2, 3, 4, 5, 6, 7, 8, and 9 cc. of water. But no mannose-hydrazone was detected in the liquor of the tenth dilution. In a similar way mannose-hydrazone was detected in the first five dilutions of the product of hydrolysis from 6 grm. of powdered date stones, but none appeared in the tests made with the sixth and subsequent dilutions. It will be noticed that the tests with ivory nut, date stones, and the flesh of cocoa nut (see below) refer only to unevaporated products of hydrolysis. In order that they may serve as standards of comparison, these results are printed below, under the table relating to pine wood.

In all the other experiments the tests were not confined to the unevaporated products of the hydrolyses, as above described, but similar methodical tests were made after the liquors had been evaporated. To this end the remainder of the acid filtrate — after the removal of the 10 cc. portion which had been abstracted for the foregoing tests — was carefully neutralized, or almost neutralized, with sodium hydroxide and evaporated over a water-bath to soft dryness. The residue was taken up with 10 cc. of water, the slightly acid solution was filtered, and the filter rinsed with 1 cc. of water which was added to the filtrate. One cc. fractions of the liquid thus obtained were diluted and tested precisely as described above, with the exception that 16 drops of the phenylhydrazin acetate reagent were added to each fraction, instead of the 12 drops used before, on the ground that a larger amount of mannose should naturally be expected to be present in the evaporated products than in the unevaporated. Here, again, the precipitates were examined under the microscope after two hours, and subsequently, if need were, at intervals during forty-eight hours. The results obtained by this methodical, progressive system of diluting and testing are set forth in the following table, where the words "Yes" and "No" mean that mannose was or was not detected: —

The pine needles and bark mentioned in the table were obtained as follows:

White pine needles plucked from a tree at Hingham, Mass., on December 18, 1902.

White pine needles plucked from a tree at Hingham, Mass., on February 15, 1903.

Fresh needles of the white pine, plucked from the tree June 10, 1902. The average length of these needles was 2.5 inches.

Old needles of the white pine shaken from the tree on June 5, 1902.

Dead needles of the white pine that had fallen from the tree in the spring of 1902. They were picked up from the ground beneath the tree on June 5, 1902.

Bark from the limb of the white pine tree cut August 9, 1902.

It is to be remembered that mannan had already been detected in this laboratory in the wood of certain other coniferous trees beside the white pine, viz., in the wood of the pitch pine (*P. rigida*), the Norway spruce (*Picea excelsa*), the hemlock spruce (*Tsuga Canadensis*), the Japanese larch (*Larix leptolepis*), the red cedar (*Juniperus Virginiana*), and the white cedar (*Chamæcyparis sphæroidea*), as has been set forth in Volume III, page 32, of this Bulletin.

Pine Wood and Pine Needles.

Name of the Material Examined.	**Unevaporated** Mannose-hydrazone was or was not detected in the several fractions of the *unevaporated* product of the hydrolysis.						**Evaporated** Mannose-hydrazone was or was not detected in the several fractions of the *evaporated* product of the hydrolysis.									
	1 cc. not diluted.	1 cc. and 1 cc. water.	1 cc. and 2 cc. water.	1 cc. and 3 cc. water.	1 cc. and 4 cc. water.	1 cc. and 5 cc. water.	1 cc. not diluted.	1 cc. and 1 cc. water.	1 cc. and 2 cc. water.	1 cc. and 3 cc. water.	1 cc. and 4 cc. water.	1 cc. and 5 cc. water.	1 cc. and 6 cc. water.	1 cc. and 7 cc. water.	1 cc. and 8 cc. water.	1 cc. and 9 cc. water.
White pine wood of January 9, 1903	Yes	No	No				Yes	Yes	Yes	Yes	No	No	No			
White pine wood of February 9, 1903	No	No	No				Yes	Yes	No	No	No					
White pine wood of March 9, 1903	No	No	No				Yes	Yes	Yes	No	No					
White pine wood of April 9, 1903	Yes	No	No				Yes	Yes	Yes	Yes	No	No				
White pine wood of May 9, 1903	Yes	No	No	No			Yes	Yes	Yes	Yes	No	No				
White pine wood of July 9, 1902	Yes	Yes	No	No	No		Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No
White pine wood of August 9, 1902	Yes	Yes	No	No			Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
White pine wood of late August, 1900	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	
White pine wood of September 9, 1902	Yes	No	No	No			Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	
White pine wood of October 9, 1902	Yes	No	No	No			Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	
White pine wood of November, 1902	Yes	No	No	No			Yes	Yes	Yes	Yes	Yes	No	No	No		
White pine wood of December 9, 1902	Yes	No	No				Yes	Yes	Yes	Yes	Yes	No	No			
White pine wood of December 14, 1901	Yes	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes	No	No			
Live pine needles, December	No	No	No				Yes	No	No	No	No	No				
Live pine needles, February	No	No	No				Yes	Yes	No	No						
Live pine needles, June	No						Yes	Yes	No	No	No	No				
Old pine needles shaken from tree in June	Yes															
Dead pine needles picked up on the ground in June	Yes						Yes	Yes	Yes	No	No	No	No	No	No	No
Bark of white pine	No	No	No	No			No	No	No	No	No	No				
	Unevaporated						**Unevaporated**									
Ivory nut (10 grms.)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No				
Date stones (6 grms.)	Yes	Yes	Yes	Yes	Yes	Yes	No	No								
Cocoa nut meat (22 grms.)	Yes	No	No	No	No	No	No	No	No	No						

As corroborating the evidence set forth in the table above given which relates to mannan in pine wood, attention should again be called to the results of efforts previously made to determine quantitatively the amounts of mannan contained in samples of pine wood cut respectively in late August and in the middle of December, as has been explained in the previous number of this Bulletin (Volume 3, page 36). In those trials it appeared that while 3.61 per cent. of mannose was obtained from the August wood, only 1.86 per cent. was yielded by the wood of the tree cut in December. Even those preliminary, isolated trials went to show that pine trees contain much more mannan in late summer than they do in early winter, and this inference has been borne out by new trials, with these particular samples of woods, made by the new method of testing and reported in the table under the dates "late August, 1900," and December 14, 1901. Manifestly, this inference has been strengthened greatly by the whole series of results obtained by the new method of testing as given in the table.

There can, I think, be little doubt that when properly studied the fact that pines, and presumably other coniferous trees, contain in late summer and early autumn a large store of the reserve food mannan may serve to explain a number of points relating to the physiology of coniferous evergreens that are now somewhat obscure. Possibly it may be true that this store of reserve food is used directly for the formation of new leaves even in the autumn to supply the place of those which are to fall at that season; or the mannan may serve for the nourishment and growth during winter of those leaves which with the advent of spring are to push off the old leaves which are then seen to fall. The very fact that the coniferæ are "evergreen" in rigorous climates may perhaps depend on the power of their leaves, when cold weather sets in, to draw upon the store of reserve matter that was accumulated by the tree during the summer.

There is one apparently well established rule of horticultural practice — widely held in England and in the Northern United States — that the best time to transplant coniferous trees is in the early autumn, which may perhaps depend absolutely on the abundance of mannan in such trees at that season. I have noticed even that one prominent nursery man in the vicinity of Boston advertises in the newspapers that "August is the best month in the year for transplanting evergreen trees with safety." Another tenet of horticultural practice has been laid down by an expert in the following terms: "The coniferæ are readily raised from cuttings which strike root best when of the present season's growth taken off near where it proceeds from the old wood and when nearly ripe, sometime in August."

It would be of interest to determine by analysis whether the marked enfeeblement of evergreen trees after they have borne a heavy crop of seeds is coincident with a diminution of the amount of mannan stored in the wood of the tree. I have noticed that, for the purpose of avoiding this enfeeblement, gardeners sometimes take pains to pick off the cones from young Norway spruces as soon after their appearance as may be practicable.

Tests similar to those above described were made upon the products of hydrolysis of other kinds of woods (and of several roots and fruits) in a somewhat desultory way, though usually the purpose of the experiments was to test some special hypothesis; such, for instance, as the question: Is mannan present or abundant in the wood of those deciduous trees which habitually and quickly put forth foliage early in the spring? To this question negative answers were returned, for the most part. Again, is there any mannan in the wood of shrubs which are evergreen though not coniferous? In so far as this question has been tested only negative answers have been obtained.

Other questions were as follows: Is there any mannan in the leaves and roots of leguminous plants, as there is in the seeds of such plants? And is there any mannan in the wood of leguminous trees? In answer to these questions, mannan was detected in the wood of two such trees, as will be seen in the table relating to woods. It was found also in the roots of red clover. Yet another question tested was to determine whether it can be said generally that there is mannan in those kinds of woods which best resist decay, as locust wood does? Here, again, the answers were negative. The following list contains descriptions of the several specimens of deciduous woods that were subjected to hydrolysis, and the table shows the results of tests made with the liquors that were obtained: —

1. Horse-chestnut wood (*Aesculus hippocastanum*). Limb 0.5 inch in diameter, cut from an old tree at Scituate, Mass., November 25, 1902.

2. Apple-tree wood (*Pyrus malus*). Limb 1 inch in diameter, cut from a tree at the Bussey Institution, September 18, 1902.

   A. The product of the hydrolysis of 22 grm. of the air-dried wood gave no distinct indication of mannose, though some suspicious globules were noticed in the first test both of the evaporated and the unevaporated liquors.

   B. Hence another hydrolysis was made, the results of which gave no positive results.

3. Linden wood (*Tilia Europæa*). Limb 1.5 inch in diameter, cut from a tree at the Bussey Institution, November 10, 1902.

4. Purple Lilac wood (*Syringa vulgaris*). A stem 0.25 to 0.50 inch in diameter, collected at the Bussey Institution, September 18, 1902.

5. Chestnut wood (*Castanea vesca*, the common American variety). Limb 1.75 inches in diameter, cut from a tree at Hingham, Mass., March 9, 1903.

6. Sassafras wood (*Sassafras officinale*). Limb 1.5 to 1.75 inches in diameter, cut from a tree at Hingham, Mass., March 28, 1903.

7. Black Wild Cherry, "Rum Cherry" (*Prunus serotina*). Limb 1 inch in diameter, cut from a tree at the Bussey Institution, September 18, 1902.

8. Box Elder wood (*Negundo aceroides*). Limb 1.75 to 2 inches in diameter, cut from a tree at Plymouth, Mass., November 8, 1902.

9. Kentucky Yellow Wood (*Cladrastis tinctoria*). Limb 0.9 inch in diameter, cut from a tree at the Bussey Institution, October 3, 1902.

10. Honey Locust wood (*Gleditschia triacanthos*). Limb 1 inch in diameter, cut from a tree at the Bussey Institution, February 25, 1903. Mannan had previously been detected in the *seeds* of Gleditschia triacanthos by Goret. Abstract in Hoffmann's Jahresbericht der Agrikulturchemie, 1900, page 210.

11. Common Locust wood (*Robinia pseudacacia*). Limb 1.25 inch in diameter, cut from a tree at Hingham, Mass., March 1, 1903.

12. Mulberry wood (*Morus alba*). Limb 2.5 inches in diameter, that showed 21 annual rings, cut from an old tree at Hingham, Mass., November 1, 1902. Only the sap wood of this specimen was examined.

13. Rhododendron maximum. Wood of a stem half an inch in diameter; cut April 2, 1903, at Reading, Mass.

14. Mountain Laurel wood (*Kalmia latifolia*). Stem 0.25 to 0.40 inch in diameter, collected early in April, 1903, at Reading, Mass.

15. Mahonia wood. Stem 0.25 to 0.05 inch in diameter, collected early in April, 1903, at Reading, Mass.

16. Andromeda. Wood of a stalk 0.25 to 0.4 inch in diameter, collected early in April, 1903, at Reading, Mass.

17. Ilex crenata. Wood of a stalk 0.25 to 0.75 inch in diameter, collected early in April, 1903, at Reading, Mass.

For the specimens Nos. 13, 14, 15, 16, and 17, of non-coniferous evergreen shrubs, I am indebted to the courtesy of Mr. A. Chandler Manning of the Reading Nursery, Reading, Mass.

Name and Number of the Specimen of Wood.	Unevaporated Mannose was or was not detected in the several fractions of the *unevaporated* product of the hydrolysis.				Evaporated Mannose was or was not detected in the several fractions of the *evaporated* product of the hydrolysis.							
	1 cc. not diluted.	1 cc. and 1 cc. water.	1 cc. and 2 cc. water.	1 cc. and 3 cc. water.	1 cc. not diluted.	1 cc. and 1 cc. water.	1 cc. and 2 cc. water.	1 cc. and 3 cc. water.	1 cc. and 4 cc. water.	1 cc. and 5 cc. water.	1 cc. and 6 cc. water.	1 cc. and 7 cc. water.
1. Horse chestnut .	No	No	No		No	No	No	No	No	No		
2. Apple *A* . . .	Tr.?	No	No	No	No	No	No	No	No	No	No	No
2. Apple *B* . . .	No	No	No		No	No	No	No	No			
3. Linden	No	No	No		No	No	No	No	No	No		
4. Purple lilac . . .	No	No	No	No	No	No	No	No	No	No		
5. Chestnut	No	No	No		No	No	No	No				
6. Sassafras	No	No	No		No	No	No	No				
7. Bl'k or Rum Cherry	No	No	No		No	No	No	No				
8. Box elder	No	No	No		Yes	No	No	No	No			
9. Ky. Yellow Wood	No	No	No	No	Yes	No	No	No	No			
10. Honey Locust . .	No	No	No		Yes	No	No	No				
11. Common Locust .	No	No	No		No	No	No	No				
12. Mulberry :												
A. 22 grm. taken	No	No	No	No	No	No	No	No	No	No		
B. 43 "	No	No	No		Yes	No	No	No	No	No		
13. Rhododendron . .	No	No	No		No	No	No	No				
14. Mountain Laurel .	No	No	No		No	No	No	No				
15. Mahonia	No	No	No		No	No	No	No				
16. Andromeda . . .	No	No	No		No	No	No	No				
17. Ilex	No	No	No		No	No	No					

In addition to the woods of the deciduous trees enumerated in the above table, tests had previously been made of the wood of the gray birch (*Betula populifolia*), of the willow (*Salix alba*), the poplar (*P. tremuliformis*), and the so called leather wood or moose wood (*Dirca palustris*), with negative results in every instance. In the products of the hydrolysis of the wood of the sugar maple (*Acer saccharinum*), however, mannose was found. See Bulletin of the Bussey Institution, Volume III, pages 32 and 34.

Tests of the products of the hydrolysis of certain leaves, other than those of pine trees, are set forth in the following list and table : —

Red Clover leaves (*Trifolium pratense*). Collected at the Bussey Institution on July 17, 1902.

Apple leaves. Plucked from a tree at the Bussey Institution, September 18, 1902.

Lilac leaves. Plucked from a bush at the Bussey Institution, September 18, 1902.

Mulberry leaves. Plucked from a tree at Hingham, Mass., September 29, 1902.

Rhododendron leaves. Leaves of *Rhododendron maximum.* Collected early in April, 1903, at Reading, Mass.

Lettuce leaves (*Lactuca sativa*). Several "heads" of lettuce, bought in Boston market, April 6, 1903, were chopped fine, after removal of the stalks, dried and ground to powder.

The tender leaf stalks of the common garden rhubarb (*Rheum rhaponticum*); as used for making pies, tarts, and sauce. Bought in Boston market, May 12, 1903.

Rock weed (*Ascophyllum nodosum*) (Fucus nodosus of Linneus) gathered from rocks at White Horse Beach, Manomet, Mass., July 21, 1902.

Name of Material Examined.	**Unevaporated** Mannose hydrazone was or was not detected in the several fractions of the *unevaporated* product of the hydrolysis.				**Evaporated** Mannose hydrazone was or was not detected in the several fractions of the *evaporated* product of the hydrolysis.						
	1 cc. not diluted.	1 cc. and 1 cc. water.	1 cc. and 2 cc. water.	1 cc. and 3 cc. water.	1 cc. not diluted.	1 cc. and 1 cc. water.	1 cc. and 2 cc. water.	1 cc. and 3 cc. water.	1 cc. and 4 cc. water.	1 cc. and 5 cc. water.	1 cc. and 6 cc. water.
Red Clover leaves	No	No	No	No	No	No	No	No	No	No	
Apple leaves	No	No	No	No	No	No	No	No	No	No	
Lilac leaves	No	No	No	No	No	No	No	No	No	No	
Mulberry leaves	No	No	No	No	Yes	No	No	No	No	No	
Rhododendron leaves	No	No	No		No	No	No	No			
Lettuce leaves	No	No	No		Yes	Yes	No	No			
Rhubarb leaf stalks	No	No	No		Yes	No	No	No			
Rock weed	Query *				?	No	No	No	No	No	No

* Some large opaque globules, insoluble in glacial acetic acid, were obtained in the case of the rock weed. They much resembled mannose-hydrazone globules, but not enough so to permit of certainty.

Of fleshy fruits only apples, olives, and bananas were subjected to hydrolysis, as will appear below.

Apple Fruit. Flesh of Baldwin apples. Three specimens were selected at different times by Mr. Hersey from a store kept in barrels at his farm at Hingham in an ordinary cellar of even temperature.

A. Specimen chosen on October 17, 1902. The apples were hard and firm and not fully "ripe."

B. Specimen chosen on February 15, 1903. The apples were now in excellent condition for eating.

C. Specimen chosen on April 13, 1903. The apples were sound and in fairly good condition, though much more mellow than those selected on February 15. From the commercial point of view they were still in passable condition, though it would not have been prudent to try to keep them any longer.

Flesh of the Banana (*Musa sapientum*). The yellow variety. Bought in Boston market May 29, 1903. The skin was not examined.

The fleshy part of pickled olives (*Olea Europæa*), bought in Boston market. After having been cut into thin slices, the flesh was dried and ground to a soft, somewhat pasty mass which was hydrolyzed with hydrochloric acid of 5 per cent. in the usual way.

According to the encyclopædias, pickled olives are the unripe fruit of a subvariety called *O. oblonga* by Duhamel. After soaking in water that contains some potash and lime, to remove bitterness, the fruit is bottled in salt and water with or without the addition of some aromatic substance.

The saline, slightly acid liquor, poured out from the bottle in which the olives had been preserved, was tested for mannose with the result that no reaction was obtained from the unevaporated liquor, while good crystals of mannose-hydrazone were obtained on evaporating the liquor to soft dryness, treating the residue with 10 cc. of water and adding phenylhydrazin acetate to three several fractions of this solution, one of 1 cc., one of 3 cc., and one of 6 cc. concentrated to 3 cc. Crystals of mannose-hydrazone appeared in each instance.

Name of the Material.	**Unevaporated** Mannose was or was not detected in the several fractions of the *unevaporated* product of the hydrolysis.				**Evaporated** Mannose was or was not detected in the several fractions of the *evaporated* product of the hydrolysis.							
	1 cc. not diluted.	1 cc. and 1 cc. water.	1 cc. and 2 cc. water.	1 cc. and 3 cc. water.	1 cc. not diluted.	1 cc. and 1 cc. water.	1 cc. and 2 cc. water.	1 cc. and 3 cc. water.	1 cc. and 4 cc. water.	1 cc. and 5 cc. water.	1 cc. and 6 cc. water.	1 cc. and 7 cc. water.
Apple flesh, *A*, Oct. 17	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes	No	No	
" *B*, Feb. 15	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	No	No	No
" *C*, Apr. 13	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Tr.	No	No	
Flesh of the Banana .	Yes	No	No		Yes	Yes	Yes	Yes	No			
Flesh of olives (pickled)	No	No	No		Yes	No	No	No				

After hydrolysis, the edible parts of several kinds of nuts, enumerated in the following list, were tested with results given in the table.

Chestnuts. Fruit of *Castanea vesca*, the ordinary, small American variety. Bought in Boston market, November 20, 1902. The shells of the nuts were discarded.

Almonds. Fruit of *Amygdalus communis.* Bought in Boston market, November 28, 1902. Only the actual meat of the almonds was examined.

Peach meats, *i.e.*, the kernels taken from peach stones (*Prunus Persica*).

Acorns. Fruit of the Scarlet oak (*Quercus coccinea*). Collected at Scituate, Mass., December 1, 1902. The shells of the acorns were discarded.

Pea Nuts or Ground Nuts (*Arachis hypogæa*). Bought in Boston market, February 12, 1903. The shells of the nuts were discarded, and so were the thin skins also that covered the meat proper.

Brazil Nut. The seed of the fruit of *Bertholletia excelsa.* Bought in Boston market, February 12, 1903. Only the firm, white meat of the nut was taken.

Filberts (European) (*Corylus Avellana*). Bought in Boston market, February 12, 1903. The shells of the nuts were discarded.

Name of the Material Examined.	**Unevaporated** Mannose was or was not detected in the several fractions of the *unevaporated* product of the hydrolysis.				**Evaporated** Mannose was or was not detected in the several fractions of the *evaporated* product of the hydrolysis.					
	1 cc. not diluted.	1 cc. and 1 cc. water.	1 cc. and 2 cc. water.	1 cc. and 3 cc. water.	1 cc. not diluted.	1 cc. and 1 cc. water.	1 cc. and 2 cc. water.	1 cc. and 3 cc. water.	1 cc. and 4 cc. water.	1 cc. and 5 cc. water.
Chestnuts	Yes	No	No	No	Yes	Yes	No	No	No	No
Almonds	No	No	No	No	Yes	Yes	No	No	No	No
Meats from peach stones	No	No	No	No	Yes	No	No	No	No	
Acorns A	No?	No	No	No	Yes	Yes	No	No	No	No
Acorns B	No	No	No		Yes	Yes	Yes	No	No	
Pea Nuts	No	No	No		Yes	No	No	No		
Brazil Nuts	No	No	No	No	Yes	No	No	No		
Filberts	No	No	No		Yes	No	No	No		
Coffee	Yes	No	No		Yes	Yes	Yes	Yes	No	
Stones of olives	No	No	No		No	No	No	No		

In the cases of the filberts, peanuts, and Brazil nuts good crystals of mannose hydrazone were obtained from the undiluted, evaporated liquor, though it was evident that no very large quantities of mannose were present in the products of the hydrolyses.

Coffee (*Coffea Arabica*). A mixture of raw "Mocha and Java" coffee, bought in Boston market. The so called coffee beans of commerce are really the seeds of the plant taken from the fleshy fruit or berry. These seeds were ground to powder and hydrolyzed in the usual way. No difficulty was encountered on testing the unevaporated product of the hydrolysis, but on evaporating the reddish liquor to soft dryness and taking up the residue with water the solution was found to be so strongly colored that it was wholly unfit for testing. The difficulty was overcome by mixing a small quantity of freshly prepared zinc hydroxide with the partially evaporated solution and filtering off the brown precipitate that fell. The evaporation of the filtrate was then proceeded with in the usual way, and several instalments of crystals of mannose-hydrazone were obtained from it, as is stated in the table.

Stones of Olives (*Olea Europæa*). From a jar of pickled olives bought in Boston market. The stones were cut open and the meats removed. Not enough of the meat was obtained to permit of its examination.

Tests of the products of the hydrolysis of roots of several kinds are enumerated in the following list and table. It should be said that mannose had previously been detected in this laboratory in the products of the hydrolysis of the roots of asparagus (*Asparagus officinalis*), chicory (*Cichorium intybus*), the Jerusalem artichoke (*Helianthus tuberosus*), and the dandelion (*Taraxacum officinale*). See Bulletin of the Bussey Institution, III, 33, 34.

Roots of the common buttercup (*Ranunculus bulbosus*) dug up at the Bussey Institution, October 25–27, 1902.

Roots of Milk weed (*Asclepias Cornuti*). Dug up at the Bussey Farm, July 27, 1902.

Roots of the large White Belgian Carrot (*Daucus carota*). Taken from a heap stored at the Bussey Farm, February 18, 1903.

Roots of red clover (*Trifolium pratense*) dug up on July 17, 1902, at the Bussey Institution.

Name of the Material Examined.	**Unevaporated** Mannose was or was not detected in the several fractions of the *unevaporated* product of the hydrolysis.				**Evaporated** Mannose was or was not detected in the several fractions of the *evaporated* product of the hydrolysis.					
	1 cc. not diluted.	1 cc. and 1 cc. water.	1 cc. and 2 cc. water.	1 cc. and 3 cc. water.	1 cc. not diluted.	1 cc. and 1 cc. water.	1 cc. and 2 cc. water.	1 cc. and 3 cc. water.	1 cc. and 4 cc. water.	1 cc. and 5 cc. water.
Buttercup roots	No	No	No	No	Yes	Yes	Yes	No		
Milk weed roots	No	No	No		Yes	Yes	Yes	No	No	
Carrot roots	Yes	No	No	No	Yes	Yes	Yes	Yes	No	No
Red clover roots	No				Yes	Yes	No	No		
Sweet potato	No	No	No	No	Yes	Yes	No	No		
Dahlia roots	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No
Sweet-flag root-stalks	No	No	No		Yes	Yes	No	No		
Sweet-flag rootlets	No	No	No		Yes	No	No			

Roots of Dahlia. From gardens at the Bussey Institution and at Hingham, Mass.

Sweet Potato (*Ipomœa batatas*). Bought in Boston market in May, 1903. Only the flesh proper was examined, after removal of the skin.

Roots, *i.e.* root-stalks of Sweet-flag (*Acorus calamus*). Dug up not far from the Bussey Institution, May 20, 1903.

Rootlets of Sweet-flag, cut off from beneath the root stalks of the foregoing paragraph.

With regard to the method of testing, in all of the foregoing experiments, it will be noted, in those cases where only negative results were obtained, that it is possible that positive results might have been got if quantities of the materials larger than 20 grams had been operated upon. The comparatively small quantity (20 grams) was chosen and adhered to because the plan of the research was merely to ascertain in a general way what kinds of plants carry an abundance of mannan, and at what seasons of the year, as distinguished from plants that contain little or none of it.

Mannose Forms in Presence of Alkalies.

Another point to be emphasized is a certain risk that some mannose might possibly be formed from the dextrose in the products of the hydrolyses unless extreme care were exercised in the process of neutralizing these products by means of sodium hydroxide, as a preliminary to their evaporation. Lobry de Bruyn * has shown that dextrose when subjected to the action of alkalies is readily changed in some part to mannose, and since dextrose must necessarily be present in the products of the hydrolysis of wood which contains cellulose, it would manifestly be improper to leave any free alkali in contact with these products of hydrolysis during the process of concentrating them by evaporation. If any free alkali were present some mannose would be formed from the dextrose, and would be exhibited as a matter of course on the addition of phenylhydrazin acetate.

In the series of tests of woods, etc., recorded in this article, special pains were taken to avoid making the products of hydrolysis actually alkaline by means of sodium hydroxide, and to have the liquids neutral or even very slightly acid, in some cases, at the beginning of the evaporation. Practically, it was found that the residues left after the evaporations exhibited an acid reaction on being taken up with water.

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* Recueil des Travaux Chimiques des Pays Bas, 1895, **14.** 156; and Lobry de Bruyn and Ekenstein, *ibid.* **14.** 203, **18.** 147.

The following experiments were made for the purpose of studying the amount of influence which may be exerted by the presence of a small quantity of alkali in a dextrose solution, and of learning whether in actual practice there is any probability that the accuracy of the test for mannose might be impaired in case the neutralization of the products of the hydrolyses were to be pushed too far.

Tests, based on Lobry de Bruyn's * original experiment, were made as follows: One gram of pure dextrose (from Merck & Co.) was dissolved in a very small quantity of water and 1.4 grm. of potassium hydroxide were dissolved in 25 cc. of water. To the dextrose solution 5 cc. of this normal potash solution were added and the resultant mixture, amounting to 10 cc., was divided into two equal portions, each of which contained 2.5 cc. of the normal potash solution, *i. e.*, 0.14 grm. of KOH. One of these portions was heated on a water-bath for an hour and a half at a temperature of 70° C. and was then nearly but not quite neutralized with hydrochloric acid of 5 per cent. The slightly alkaline liquid was evaported at a temperature of 70° to soft dryness, and the residue was taken up with 1 cc. of water. To this solution 20 drops of the phenylhydrazin acetate reagent were added. A brown precipitate that contained much amorphous matter formed at once. After it had stood for two hours mannose-hydrazone balls were seen in it with the microscope, and on recrystallizing from warm diluted alcohol (three parts of alcohol of 95 percent. and one part of water) good crystals and plates of mannose-hydrazone were obtained. The other portion of mixed dextrose and potash was heated for an hour and a half on a boiling water-bath, then nearly neutralized with hydrochloric acid of 5 percent. and evaporated to soft dryness on the water-bath. The residue was taken up with 1 cc. of water and the solution mixed with 20 drops of the phenylhydrazin acetate reagent, but a far less satisfactory result than that of the foregoing experiment was obtained. Only a few globules, somewhat resembling mannose-hydrazone balls, appeared in the amorphous precipitate, and no crystals were got on trying to recrystallize the balls.

* Recueil des Travaux Chimiques des Pays Bas, 1895, **14.** 158–161, 204.

In another trial half as much potash was used as before: 0.5 grm. of the pure dextrose was dissolved in a small quantity of water and to it were added 1.25 cc. of the normal solution of potash (*i. e.*, 0.07 grm. KOH). The mixed solution was made up to 5 cc. and heated on a water-bath for an hour and a half at a temperature of about 70°. Hydrochloric acid of 5 percent. was added to slight acidity and then a few drops of potash solution, enough, that is to say, to make the liquid very faintly alkaline. The solution was evaporated to soft dryness, the residue was taken up with 1 cc. of water and the solution mixed with 16 drops of the phenylhydrazin acetate reagent. After a few hours many mannose-hydrazone balls were visible in the precipitate, and excellent crystals of this substance were obtained on recrystallizing the balls from diluted alcohol.

Another test was made using half as much potash as in the last trial: To 0.5 grm. of the pure dextrose, dissolved in a small quantity of water, there was added 0.625 cc. of a normal solution of potash (*i. e.*, 0.035 grm. of KOH). The mixed liquor amounting to about 5 cc. was heated on a water-bath during an hour and a half at a temperature of 70° C. The liquid was acidified with a few drops of hydrochloric acid of 5 percent., then made very faintly alkaline with potash and evaporated to soft dryness. The residue was taken up with 1 cc. of water, and there was added to it 16 drops of the phenylhydrazin acetate reagent. A brownish precipitate formed slowly, and there was seen in it after a few hours many large mannose-hydrazone balls, the number of which increased on longer standing. On recrystallizing from diluted alcohol excellent crystals of mannose-hydrazone were obtained.

A test in which half as much potash was used as in the last case was as follows: To 0.5 grm. of the pure dextrose, dissolved in a small quantity of water, 0.31 cc. of the normal potash solution was added (*i. e.*, 0.017 grm. of KOH). The liquid was made up to the volume of 5 cc. and heated on a water-bath at 70° during an hour and a half. A few drops of hydrochloric acid of 5 percent. were then added, but not enough to fully neutralize the solution which was left very faintly alkaline. In this condition it was evaporated to soft dryness. The residue was taken up with 1 cc. of water and the solution mixed with 16 drops of the phenylhydrazin acetate reagent. A reddish-brown precipitate formed slowly. After four hours there were seen in it some spiny crystals of dextrose osazone and a very few large, yellowish globules which may have been mannose-hydrazone. Next day numerous mannose-hydrazone balls, both large and small, were visible, and excellent crystals of this substance were obtained by recrystallizing from diluted alcohol.

Yet another trial was made, using half as much potash as in the preceding instance: To 0.5 grm. of the pure dextrose, dissolved in a small quantity of water, 0.15 cc. of normal potash solution was added (*i.e.*, 0.008 grm. of KOH). The liquid, amounting to about 5 cc., was heated on a water-bath at the temperature of 70° C. during an hour and a half. It was then found to have an alkaline reaction and was made slightly acid by adding a few drops of hydrochloric acid of 5 percent. Next it was made slightly alkaline by the addition of a drop or two of the normal potash solution and was evaporated to soft dryness. The residue was taken up with 1 cc. of water and 16 drops of the phenylhydrazin acetate solution were added. A reddish-brown precipitate formed slowly and some spiny crystals of dextrose osazone were seen in it. There were a few yellowish balls also which may perhaps have been mannose-hydrazone, but this point was uncertain, and only dextrose osazone crystals were obtained on recrystallizing from diluted alcohol.

It was argued that the quantity of potash used in the last but one of the above trials probably corresponded somewhat nearly to the quantity of soda that might be present in the ordinary method of testing for mannose in woods in case the acid product of the hydrolysis had been decidedly over-neutralized with sodium hydroxide. To test this idea, 0.4 grm. of the pure dextrose was dissolved in 20 cc. of water, a few drops of strong hydrochloric acid were added, and the mixture was left at rest during forty-eight hours. Enough of a solution of sodium hydroxide was then added to make the solution decidedly alkaline. A part of the solution was examined without having been concentrated by evaporation, while the remainder was evaporated to soft dryness. The residue was taken up with 4 cc. of water. As regards the unevaporated liquor, 12 drops of the phenylhydrazin acetate reagent, added to 1 cc. of the liquor, gave a small yellowish precipitate in which some spiny crystals of dextrose-osazone appeared, but there were no indications of mannose-hydrazone. In the case of the evaporated liquor, 16 drops of phenylhydrazin acetate reagent, added to 1 cc. of the liquor, gave a yellowish-brown precipitate, that formed slowly, in which after three hours a few crystals of dextrose-osazone were seen, and some balls insoluble in glacial acetic acid which were presumed to be mannose-hydrazone. On standing over night both the mannose-hydrazone balls and the spiny osazone crystals had increased, and on recrystallizing from diluted alcohol good mannose-hydrazone crystals were obtained.

To test the effect of dilution, 1 cc. of water was added to 1 cc. of the liquor from the evaporation, and then 16 drops of the phenylhydrazin acetate solution. Next day the precipitate was seen to contain many crystals of dextrose-osazone and a few mannose-hydrazone balls. On again diluting, with 2 cc. of water, 1 cc. of the liquor from the evaporation and adding 16 drops of the phenylhydrazin acetate reagent and allowing the mixture to stand over night, there were found a few dextrose-osazone crystals and some mannose-hydrazone balls also.

But in other mixtures of dextrose and alkali made to illustrate what might happen in case the over neutralization of the product of an hydrolysis was but slight, no indications of mannose-hydrazone were detected. Thus, when 0.4 grm. of the pure dextrose, dissolved in 20 cc. of water and acidified with a few drops of strong hydrochloric acid, left at rest during twenty-four hours, then neutralized with sodium hydroxide to slight alkalinity and mixed with 12 drops of the phenylhydrazin acetate reagent, no indications of mannose were obtained, neither on testing the unevaporated liquor as it stood or after diluting it with water. Only amorphous precipitates and some dextrose-osazone crystals were to be seen after long standing. Similar negative results were encountered on evaporating the dextrose liquor to soft dryness, taking up with 4 cc. of water and adding 16 drops of the phenylhydrazin acetate reagent to 1 cc. of the liquor. No mannose-hydrazone but an abundance of dextrose-osazone crystals were visible after the mixture had stood for a number of hours. So, too, on diluting with water some portions of the liquor from the evaporation, before adding the phenylhydrazin acetate reagent, only dextrose-osazone crystals, but no mannose-hydrazone were obtained.

In another trial, one gram of the pure dextrose was dissolved in a small quantity of water and 5 cc. of a normal solution of potash were added. The volume of this mixed solution was about 12 cc. One half of it was heated on a water-bath for an hour and a half to 70° C., and the other half was heated on a boiling water-bath for the same length of time. Both the liquors took on a deep red color and that on the boiling bath had the odor of burnt sugar. Both the liquors were nearly but not quite neutralized with hydrochloric acid of 5 percent., and each of them was mixed with 25 drops of the phenylhydrazin acetate reagent. No indications of mannose-hydrazone were obtained in either case from these unevaporated liquids, probably because the solutions were too dilute to permit mannose-hydrazone to separate out. It will be noticed that in one of the experiments above recorded, which closely resembled this one, no indications of mannose were got when the unevaporated solution was tested, though good balls and crystals of mannose-hydrazone were obtained when the evaporated solution was examined.

Of course, the conditions that obtained in this series of experiments, where pure dextrose was brought into contact with weak alkaline solutions, differed considerably from those of actual practice where the dextrose in the products of the hydrolyses was commingled with organic matters of various and unknown characters. It is conceivable perhaps that some of these extraneous substances might act to shield dextrose from the influence of the alkali, but the experiments go to show clearly enough that in testing for mannose extreme care must be exercised to avoid the action of alkalies in all cases where dextrose is known to be present.

No Mannose was detected in Maple Sugar.

The presence of mannan in the wood of sugar maple trees, as set forth on pages 32–34 of Vol. III of the Bulletin of the Bussey Institution, suggested the inquiry whether the sugar prepared from the sap of such trees in the spring might not contain some mannose. The following experiments made to test this question gave only negative results, though for the sake of completeness it might perhaps be well to make yet another trial, and to test the actual sap as obtained directly from the trees.

Samples of maple sugar, maple syrup, and a magma of sugar and syrup that was practically a molasses, were procured from a source which ensured the purity of these products. The samples were known to be genuine and unmixed with sugar from any other source than the maple tree. In the case of the sugar, lumps as large as small chestnuts were dissolved in 2 cc. of water and to some of these solutions were added 12 drops of the solution of phenylhydrazin hydrochlorate and sodium acetate described on page 14 of the previous number of this Bulletin, while to others were added 12 drops of the ordinary phenylhydrazin acetate reagent. No indications of mannose-hydrazone were detected in any of these trials.

In the case of the syrup, one test was made by mixing together 1 cc. of the syrup, 1 cc. of water, and 12 drops of the solution of phenylhydrazin hydrochlorate and sodium acetate just now alluded to; another test was made by mixing 1 cc. of the syrup, 1 cc. of water, and 12 drops of the ordinary phenylhydrazin acetate reagent, and yet another by mixing 5 cc. of the syrup with 1 to 2 cc. of the phenylhydrazin acetate reagent. No indications of mannose-hydrazone were obtained in either of these tests.

In the case of the molasses also, one test was made by adding to 1 cc. of the liquid portion of the molasses, 1 cc. of water, and 16 drops of the phenylhydrazin hydrochlorate and sodium acetate solution above alluded to; and other test was made by mixing 1 cc. of the liquid molasses, 1 cc. of water, and 16 drops of the ordinary phenylhydrazin acetate reagent. In neither instance was any indication of mannose-hydrazone detected.

Much more elaborate tests both of the syrup and the molasses were then made in a different way, and in some instances much larger quantities of the materials were operated upon. As regards the syrup proper, some 25 to 30 cc. of it were treated with a few drops of basic lead acetate, prepared according to Parrish, as mentioned in a note on page 42 of the previous number of this Bulletin, and the heavy, white gelatinous precipitate was collected and washed with cold water. To the filtrate from this first precipitate more of the basic lead acetate was added and the new gelatinous, white precipitate was collected and washed in its turn. No new precipitate fell on adding the basic acetate of lead to the filtrate from the second precipitate. Each of the precipitates was covered with dilute sulphuric acid and allowed to stand over night before filtering. The filtrates from the lead sulphate were neutralized carefully with dilute ammonia and evaporated to thick syrups upon water-baths. The residues were taken up with 10 cc. of a mixture of equal parts of alcohol of 95 percent. and water; the solutions were filtered, warmed to expel the alcohol, and tested with the phenylhydrazin acetate reagent. No mannose-hydrazone was obtained neither directly nor on trying to recrystallize the precipitates by means of warm alcohol.

A much larger quantity of the syrup, amounting to some 500 cc., was treated with the basic lead acetate, and three successive precipitates were obtained. They were decomposed separately by passing carbonic acid gas through water in which they were suspended. The filtrates from the lead carbonate were evaporated to dryness, the residues were taken up with diluted alcohol (3 : 1) and evaporated to syrups which were mixed with water and, after filtration, tested with the phenylhydrazin acetate reagent. No mannose-hydrazone was obtained.

As regards the molasses, so called, the liquid portion of the magma was allowed to drain away from the solid particles of sugar, and the thick syrup thus obtained was diluted with twice its volume of water. Meanwhile, the solid sugar from which the thick syrup had drained away was dissolved in 500 cc. of water and commingled with the diluted syrup just now mentioned. The volume of the mixture amounted to about 800 cc. To it were added 1 or 2 cc. of the solution of basic lead acetate. The heavy, brownish-white precipitate produced was filtered off, washed with water, and decomposed by means of a current of carbonic acid. After the lead carbonate had been removed by filtration, the liquid was evaporated to dryness on a water-bath. The residue was taken up with 3–5 cc. of diluted alcohol (3 : 1) that was slightly warm, and the filtrate was evaporated to a thick syrup which was taken up with 3 cc. of water; 16 drops of the phenylhydrazin acetate reagent were added to this aqueous solution and a yellowish amorphous precipitate fell at once. Some crystals of dextrose-osazone appeared in this precipitate after long standing, but no mannose-hydrazone was detected.

To the filtrate from the first precipitate produced by the basic lead acetate a new quantity of this reagent was added and a second heavy, white precipitate was obtained. It was decomposed with carbonic acid as before, and the solution was treated and tested in the same way as that obtained from the first precipitate had been treated. Some crystals of dextrose-osazone, but no indications of mannose-hydrazone were noticed. Yet another, third, precipitate obtained by means of the basic lead acetate was treated in the same way as the preceding, but no mannose-hydrazone was observed.

The three precipitates produced on the addition of the phenylhydrazin acetate reagent, as above stated, were washed with water, then put together and washed with a few drops of glacial acetic acid and recrystallized from warm diluted alcohol (3 : 1), but no indications of mannose-hydrazone were detected.

On the further addition of basic lead acetate to the filtrate from the third precipitate above mentioned, a fourth and a fifth precipitate were obtained. They were treated as the earlier precipitates had been treated, but no indications of mannose-hydrazone were seen in either instance. The five precipitates, consisting of lead carbonate, etc., as left after passing carbonic acid through the precipitates produced by basic lead acetate were ground in a mortar, together with dilute sulphuric acid, the lead sulphate was filtered off and to the filtrate sodium hydroxide was added to slight alkalinity. The liquid was evaporated to soft dryness, and the residue treated with warm diluted alcohol. The filtered alcoholic solution was evaporated to dryness, the residue was taken up with 3 cc. of water, and 16 drops of the phenylhydrazin acetate reagent were added. No indications of mannose-hydrazone were obtained.

Finally, the matter left in the can from which the molasses had been poured was dissolved in 250 cc. of water, and 2 or 3 cc. of the basic lead acetate solution were added. The heavy, white gelatinous precipitate was washed with water and decomposed with carbonic acid as before. The filtrate from the lead carbonate was evaporated to a thick syrup which was treated with diluted alcohol. The alcoholic filtrate was evaporated in its turn to a thick syrup which was taken up with 3 cc. of water; 16 drops of the phenylhydrazin acetate solution were added to this aqueous solution. There were no indications of mannose-hydrazone. A second white precipitate, obtained by adding basic lead acetate to the filtrate from the first precipitate, was treated like the first precipitate, but no indications of mannose-hydrazone were manifested.

These negative results, obtained on testing maple sugar and maple syrup, agree with those of Pellet,* who has declared that fresh cane juice contains no mannose, but they differ decidedly from those published by several chemists who have tested molasses derived from the sugar-cane.†

The reason of this apparent discrepancy is that in the making of sugar from the sugar-cane the alkali lime is used for clarifying the cane juice, and that some mannose is formed by the action of this lime upon dextrose contained in that juice. But in the process of preparing sugar from the sap of the maple tree lime is seldom or never used, and there can consequently be very little chance of encountering any mannose that has been introduced into the product in that way. It may be remarked, furthermore, in passing, that Fischer and Hirschberger were unable to detect mannose either in honey or in grape juice. See abstract in Hoffmann's Jahresbericht der Agrikulturchemie, 1888, **31.** 331.

I have yet again to thank my assistant, Mr. F. T. Dillingham, most heartily for aid in this research.

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* Bulletin de l'Association des Chimistes de Sucrerie et de Distillerie de France et des Colonies, April, 1901, **18.** No. 10, page 758.

† See, for example, Lobry de Bruyn and Ekenstein. Recueil des Travaux Chimiques des Pays Bas, 1895, **14.** 215, **16.** 280; Prinsen Geerlig, Bulletin de l'Association des Chimistes de Sucrerie et de Distillerie de France et des Colonies, **14.** 1080; and Pellet, *ibid.* 1901, **18.** No. 10, page 758.

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No. 4. — *A supplement to the article (No. 3) on the Occurrence of Mannan in Trees, Roots, and Fruit.** By F. H. Storer, Professor of Agricultural Chemistry.

Pine wood cut in June. — To complete the series of experiments on the quantity of mannan in white pine wood at different times in the year, as set forth in the tabular statement on page 50, a limb 1¼ inches in diameter was cut from a thrifty young pine tree at Hingham, Mass., on June 9, 1903, and 22 grm. of the wood were subjected to hydrolysis with hydrochloric acid of 5 per cent. The product of the hydrolysis was treated and tested in the same way that the other specimens mentioned in the table had been treated. In this case, crystals of mannose-hydrazone were obtained, on adding phenylhydrazin acetate, in 1 cc. of the unevaporated product of the hydrolysis, but not in 1 cc. samples that were diluted respectively with 1, 2, and 3 cc. of water before adding the phenylhydrazin reagent.

After the remainder of the product of the hydrolysis had been neutralized and evaporated to dryness and the residue taken up with 10 cc. of water tests with phenylhydrazin acetate applied to 1 cc. of this solution, without further dilution, and to 1 cc. portions that were diluted respectively with 1, 2, and 3 cc. of water, all gave balls of mannose-hydrazone, but none of this substance was seen in trials where 1 cc. portions of the solution were diluted with 4 cc. and 5 cc. of water.

Pine wood that had long lain buried in a swamp. — The sample hydrolyzed was a chip cut from a large log three feet in diameter that had been dug up in Bear Swamp, Hingham, Mass., a few years previously. Mr. E. Hersey, to whose kindness I am much indebted, assures me that the tree must have fallen more than two hundred years ago and buried itself so deeply in the bog that the wood was preserved from decay. To all appearance the wood was white pine, though Mr. Hersey suggests that the tree may possibly have been an example of a somewhat apocryphal "yellow pine" that is thought by some to have grown formerly in Massachusetts and to have been neither pitch pine (*Pinus rigida*) nor red ("Norway") pine (*Pinus resinosa*).

After hydrolyzing with hydrochloric acid of 5 per cent., no crystals or balls of mannose-hydrazone were got from the unevaporated product of the hydrolysis. But after evaporation mannose-hydrazone was obtained from 1 cc. of the undiluted solution, and also from 1 cc. that was diluted with 1 cc. of water; but no mannose-hydrazone was obtained from portions that were more strongly diluted.

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* Bulletin of the Bussey Institution, 1903, **3.** 47. It may here be said that the methods of analysis and procedure adopted in this supplementary note are precisely the same as those employed in the original article (No. 3), unless otherwise expressly stated.

Pine wood that had long lain on the ground exposed to the weather. — Pieces of limbs of white pine picked up at Hingham, Mass., by Mr. Hersey, on land from which to his knowledge a grove of white pine trees had been cut off twenty-five years or more previously.

A was a limb $1\frac{1}{2}$ inches in diameter. It was much decayed, and crumbled to pieces between the fingers. It was hydrolyzed with hydrochloric acid of 5 per cent. and treated in the usual way. No mannose-hydrazone was got from the product of the hydrolysis, — neither from the evaporated or the unevaporated product.

B was a limb 2 inches in diameter. It was less decayed than sample *A*. No mannose-hydrazone was got from the unevaporated product of the hydrolysis; but from 1 cc. of the solution from the evaporated portion mannose-hydrazone balls and crystals were obtained both before dilution and after dilution with 1 and 2 cc. of water. No mannose-hydrazone was got from portions of the liquid which were more strongly diluted.

Spruce wood (*Picea nigra*), from a stem $2\frac{1}{2}$ inches in diameter, collected by my assistant, Mr. F. T. Dillingham, at Durham, New Brunswick, on August 14, 1903. After the usual treatment mannose-hydrazone was got from 1 cc. of the unevaporated product of the hydrolysis, but not from portions that were more strongly diluted. From the evaporated product of the hydrolysis mannose-hydrazone balls and crystals were got from 1 cc. of the undiluted solution, and from 1 cc. portions that were diluted respectively with 1, 2, 3, and 4 cc. of water, but not when 5 cc. of water were added.

Fir wood (*Picea balsamifera*), from a stem $1\frac{1}{2}$ inches in diameter, collected at Durham, New Brunswick, by Mr. Dillingham, on August 14, 1903. After the usual treatment, mannose-hydrazone was got from 1 cc. of the unevaporated product of the hydrolysis, but not from portions that were more strongly diluted. From the evaporated product of the hydrolysis mannose-hydrazone balls were got from 1 cc. of the undiluted solution, and from 1 cc. portions that were diluted with 1, 2, and 3 cc. of water, but not on dilution with 4 cc.

The following results of tests of sundry vegetables, etc., are to be regarded as additions to the tables already given on pages 55, 56, and 58: —

Yellow Turnip. — Bought March 17, 1904. The roots were peeled and only the flesh proper was ground to a pulp and subjected to hydrolysis. Excellent mannose-hydrazone crystals were obtained on testing 1 cc. of the unevaporated product of the hydrolysis, but none were got after diluting a similar quantity with 1 cc. of water. From 1 cc. of the solution obtained after evaporating the product of the hydrolysis, mannose-hydrazone crystals were obtained, and they were got also on diluting 1 cc. of the solution with 1, 2, and 3 cc. of water, but they were not got when the dilution was pushed to 4 cc. of water.

Cabbage. — A head of cabbage, bought on March 11, 1904, was divided into the several leaves that composed it. These leaves were dried at a moderate heat, and afterwards ground to a fine meal and hydrolyzed with hydrochloric acid of 5 per cent. On adding the phenylhydrazin acetate reagent to 1 cc. of the unevaporated product of the hydrolysis excellent crystals of mannose-hydrazone were obtained, but none were got from 1 cc. that was diluted with 1 cc. of water. From 1 cc. of the solution obtained from the evaporated product of the hydrolysis excellent mannose-hydrazone crystals were obtained, and so they were also from 1 cc. of the solution after dilution with 1 cc. of water, but none were got on diluting with 2 and with 3 cc. of water.

Beets. — Bought from a provision dealer on March 5, 1904. They were sliced, dried at a moderate heat, and ground to a fine meal before the hydrolysis. No mannose-hydrazone was detected in the unevaporated product of the hydrolysis. But good mannose-hydrazone crystals and plates were got from 1 cc. of the solution prepared after the evaporation of the product of the hydrolysis, and also from a 1 cc. portion that was diluted with 1 cc. of water, but none were found after diluting with 2 cc. of water.

Parsnips. — Bought March 5, 1904. The sliced roots were dried at a moderate heat and ground to a fine meal. From the unevaporated product of the hydrolysis no reaction for mannose-hydrazone was obtained; but after evaporation a not very abundant crop of mannose-hydrazone plates and prisms was observed on testing 1 cc. of the solution. None were found on diluting 1 cc. portions of the solution with 1 cc. and with 2 cc. of water.

Celery Stalks. — Bought on November 21, 1903. The green, terminal leaves were removed, the stalks were cut to thin slices and ground to a fine meal after having been thoroughly dried at a moderate heat. No mannose-hydrazone was detected on testing the unevaporated product of the hydrolysis. But good reactions were obtained from the solution prepared from the evaporated product of the hydrolysis. Crystals and plates of mannose-hydrazone were obtained from 1 cc. of the solution, and also from 1 cc. portions that had been diluted respectively with 1 and 2 cc. of water, but not from those diluted with 3 and 4 cc.

Prunes. — Bought April 7, 1904. The flesh was cut away from the stones, chopped fine, and ground up in a mortar and hydrolyzed with hydrochloric acid of 5 per cent. Mannose-hydrazone balls and crystals were obtained on testing 1 cc. of the unevaporated product of the hydrolysis, but none were seen on diluting another 1 cc. portion with 1 cc. of water. From 1 cc. of the solution, prepared after evaporating the product of the hydrolysis to dryness, mannose-hydrazone crystals and plates were obtained, and they were got also from 1 cc. portions that were diluted with 1 and with 2 cc. of water, but not from 1 cc. portions that were diluted with 3 and 4 cc. of water.

Figs. — Dried and pressed figs of commerce, bought May 29, 1903. After having been cut into small pieces and dried for several days, the fragments were cut into very small pieces, 22 grm. of which were rubbed up in a mortar together with enough hydrochloric acid of 5 per cent. to make a thin paste, which was poured into a flask and made up to the volume of 220 cc. by adding more of the acid. The mixture was boiled during three hours. Ten cc. of the clear filtrate were treated with sodium hydroxide to slight alkalinity, and then made faintly acid with hydrochloric acid. On adding the phenylhydrazin acetate reagent to 1 cc. of this solution excellent crystals of mannose-hydrazone were obtained, but none were got when 1 cc. portions of the solution were diluted with 1 and 2 cc. of water before adding the phenylhydrazin acetate. When the product of the hydrolysis was evaporated to soft dryness and the residue was taken up with 10 cc. of water, good crystals of mannose-hydrazone were obtained on adding the phenylhydrazin acetate to 1 cc. of the solution, and to 1 cc. portions which were diluted respectively with 1 and 2 cc. of water, but not when 1 cc. portions were diluted with 3 and 4 cc. of water.

Carrot Leaves. — Plucked on October 19, 1903. The leaves were dried at a moderate heat and ground to powder. No mannose-hydrazone was detected either in the unevaporated or the evaporated product of the hydrolysis.

June Grass (*Poa pratensis*). — Fresh, young leaves of the grass mown on May 6, 1904, when they were four inches long. The fresh grass was cut into small pieces, which were hydrolyzed directly with hydrochloric acid of 5 per cent. No mannose-hydrazone was detected either in the unevaporated or the evaporated product of the hydrolysis.

Oat Hay. — The oat plants were mown on the Bussey Farm towards the end of June, 1902, when the grain was "in the milk." The hay had been stored in a dry barn until November, 1903. As a preparation for the hydrolysis the hay was cut into small pieces and ground to a fine meal. No mannose-hydrazone was detected either in the unevaporated or the evaporated product of the hydrolysis.

Hydrolysis of Ivory Nut and Birch Wood with Nitric Acid. — For the sake of seeing what happens when ligneous matters that do or do not contain mannan are hydrolyzed with dilute nitric acid a couple of experiments were made, one with the ivory nut (*Phytelephas macrocarpa*) and one with the wood of the gray birch (*Betula populifolia*).

Clean buttons cut from the ivory nut were ground to a fine powder, and 22 grm. of this powder were boiled during three hours with 220 cc. of nitric acid of 5 per cent. Ten cc. of the filtered product of the hydrolysis were neutralized with sodium hydroxide to slight alkalinity, and were then made faintly acid with nitric acid. To 1 cc. of this solution 12 drops of the phenylhydrazin acetate were added. A yellow precipitate formed slowly. After standing for one hour it was examined with the microscope, and was seen to contain many mannose-hydrazone crystals as well as many osazone crystals with needle-like spines. On recrystallizing from 3 : 1 alcohol, there was obtained excellent clean cut crystals of mannose-hydrazone in the form of plates and prisms.

In a precisely similar trial made with nitric acid and wood of the gray birch, instead of the ivory nut, no mannose-hydrazone was detected either in the unevaporated or the evaporated product of the hydrolysis. Crystals of osazones were seen, naturally enough.

As was the case in the experiments to which this article forms a supplement, I am indebted to my assistant, Mr. F. T. Dillingham, for zealous aid in this research.

No. 5. — *Remarks on the "Popping" of Indian Corn.* By F. H. Storer, Professor of Agricultural Chemistry.

An opinion has long been prevalent, dependent on I know not whose authority, that the "popping" of Indian corn is in some way connected with the presence of oil in the grain. Thus, in the article on Indian corn in Appleton's New American Cyclopaedia,* we are told that "Pop-corn abounds in oil. . . . It is this closer and albuminous-oily structure in some kinds of maize which renders the kernel so compact and hard, and which on bursting with heat causes the popping or inversion of the contents of the grain." So, too, in the definition of the word pop-corn in the Century Dictionary,† it is said that "Pop-corn abounds in oil, the expansion of which under heat causes an explosion, in which the contents of the kernel become puffed out, nearly hiding the seed-coat, and assuming a pure white color."

This idea as to the significance of oil in the act of popping is in itself so palpably improbable that I have often wished to subject it to the test of experiment. Opportunity to do so presented itself in the autumn of 1902, when my assistant, Mr. F. T. Dillingham, was good enough to carry out under my eye a few simple experiments. The purpose of these trials was, first, to disprove the notion that popping is due to the presence of oil in the grain, and, second, to determine whether popped corn contains any more soluble starch, or other form of dextrin, than is contained in the original grain.

To remove the oil, unbroken grains of unpopped corn were leached with ether and were subsequently allowed to dry slowly in the air. After five days' drying they were popped successfully on being heated, though it appeared that time enough should be allowed to make the drying thorough. No proper success was had on trying to pop leached kernels that had lain in the air no longer than a day or two after having been removed from the ether.

With regard to the quantity of matters soluble in water that were contained in them, no great difference was noticed between popped and unpopped corn, though the popped yielded rather less than the unpopped. Thus, air-dry popped corn ground to a fine meal gave 17.86 per cent. of soluble matters on being leached with water at a temperature of 60° to 80° C., while unpopped corn similarly treated gave 18.59 per cent. On referring these figures to meal dry at 100° C., it appears that the popped corn contained 19.30 per cent. of matters soluble in water, while the unpopped corn contained 21.12 per cent.

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* Edition of 1860, volume XI, page 88, column 2.

† Edition of 1890, volume IV, page 4620.

On testing for matters capable of reducing cupric oxide no more than mere traces were detected either in the popped or the unpopped samples. These tests, made merely for the sake of comparison, were as follows: One gram of each of the finely powdered materials was stirred up with 25 cc. of cold water for one hour, the solutions were filtered, and the clear filtrates were added to a quantity of boiling Fehling's solution which was kept boiling during two minutes after the addition. No appearance of reduction or of precipitation could be seen in either of the liquors, but on filtering them through paper immediately after the boiling very small quantities of red precipitates of cupreous oxide were visible in both cases, and on comparing the two precipitates it seemed to the eye that the quantity obtained from the unpopped corn was a trifle larger than that from the popped samples. Though the difference between the two precipitates was but slight, an impression was left on the mind that the quantity of reducing sugar in the unpopped corn was, if anything, a little larger than the quantity contained in the popped material.

It was plain that after the act of popping less hydroscopic moisture was retained by the corn than was contained in it naturally, —that is, before popping. Thus, on drying at 95° to 100° C. samples of the meal from popped and unpopped corn, it appeared that the popped corn contained 7.45 per cent. of moisture, while the unpopped corn held 12.13 per cent.

As regards the question whether any soluble starch is formed during the act of popping, the evidence, in so far as it goes, is distinctly negative. A solution obtained by leaching popped corn with water at a temperature of 80° C. gave no special indications of the presence of soluble starch. Filtered while still warm and evaporated nearly to dryness before it had time to cool, the liquid left a somewhat gummy residue, but there was nothing of the nature of a powder or of a granular precipitate.

In order to see whether soluble starch could be prepared any more readily from the corn after it had been popped than before, experiments * were made as follows: —

Two or three grams of each of the fine meals — and of commercial (maize) starch also, for the sake of comparison — having been stirred up with 10 to 15 cc. of a concentrated solution of sodium hydroxide, it was noticed that thick pastes began to form in the course of a few minutes in the case of the unpopped corn and of the starch also, while no such paste was formed by the action of the alkali on the meal of the popped corn, though a paste that gave a bright blue coloration with an aqueous solution of iodine was obtained on boiling some of the popped corn meal with a small quantity of water.

The mixtures of the several materials and the sodium hydroxide were left at rest during three or four hours and then neutralized with dilute hydrochloric acid. A large excess of concentrated hydrochloric acid was next added to make the mixtures fluid, and the resulting solutions were filtered through double filters with the aid of a suction pump. It was observed that the mixture from the unpopped corn filtered much more readily than did that from the popped corn. On adding an excess of 95 per cent. alcohol to each of the filtrates, white precipitates of soluble starch began to form at once and settled out in the course of a night. After decanting off the supernatant liquors the precipitates were washed by decantation with cold water, and portions from each of them were dissolved in water at 60° to 80° C. All these solutions gave deep blue colorations on being tested with an aqueous solution of iodine. But so far from there being any evidence that soluble starch is formed when corn is popped, the appearances left an impression that rather more soluble starch was obtained from the unpopped corn than from the popped in the experiment above described.

[NOTE. — Since this article was written, I have learned through a paper published by the United States Department of Agriculture,† that Professor William H. Brewer, of New Haven, long ago disproved the notion that the oil in corn has any influence on

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* After Bondonneau, Bulletin de la Societé Chimique de Paris, 1874 (N.S.), **21.** 148.

† Experiment Station Work XXVI, Farmers' Bulletin, 1904, No. 202, p. 17.

the act of popping. He argued that "the popping is apparently due to the bursting of the starch grains. Only the flinty varieties 'pop.' These are more compact in structure, and as the starch grains expand with the heat the hard exterior is burst." *

Mr. M. I. Wilbert,† also, has insisted on the fact that the popping of corn is due to the explosion of the starch grains by steam generated within them on the application of heat.]

A more elaborate study of the causes of corn popping has been made recently by Professor Henry Kraemer,‡ of Philadelphia, who has shown by careful microscopic examinations of many varieties of maize that there are such marked differences in the structure of the several parts of the kernels that at least three distinct kinds of corn grains may be distinguished according to the character of their endosperms. In the pop-corns proper the endosperm is more or less translucent and horny, and the cells in it contain closely arranged polygonal starch grains from 7 to 18 microns in diameter and having a central rarified area from 2 to 7 microns in diameter. "On examining the endosperm of the popped grain it is observed that there has been considerable alteration in the starch grains and cell walls. . . . The structural characteristics of the starch grains in the altered areas of the different pop-corns would seem to indicate that the popping of the grain of corn results from the expansion of the individual starch grains, the degree of expansion depending upon the relative amount of water and air in the grains. As an illustration of this it may be stated that perfectly fresh pop-corn or pop-corn that has been soaked in water for twenty-four hours will pop but little in the true sense of the word. On the other hand, a pop-corn which was seven years old, but had not lost its germinating power, would not pop unless first soaked in water and then allowed to dry for from four to twelve hours. That this property probably resides in the starch grains is further shown by the fact that pieces of the pop-corn grain will pop."

This last statement of Professor Kraemer, that *pieces* of pop-corn will pop, suggested to me the following experiments: —

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* See page 103 of William H. Brewer's Report on the Cereal Production of the United States, in Tenth Census of the U. S. 1880, Statistics of Agriculture, page 483.

† American Journal of Pharmacy, 1903, **75**. pp. 77, 100.

‡ Science, May 1, 1903 (N.S.), **17**. 683.

I. The outer skins were removed from a number of kernels of pop-corn (the variety known as rice-corn) and attempts were made to pop them, but without success. On being heated, the skinned kernels merely swelled to a slight degree, but they did not burst.

II. Kernels that had not been skinned were cut in two, crosswise of the length of the kernel, and the half kernels thus obtained were subjected to the test of popping. It appeared at once that those halves of the kernels which in their natural state lay nearest the cob did not pop when heated, while the outer halves of the kernels popped readily. When the skin was removed from the kernels before cutting them in two crosswise, neither the outer nor the inner halves of the kernels could be made to pop.

III. Halves of kernels that had been cut lengthwise into two parts popped readily in some of the trials, and it did not appear in this case that the popping was confined to any particular part of the kernel.

IV. Some unskinned kernels of pop-corn were cut in two lengthwise, and the long half kernels thus obtained were cut in two crosswise, so that each kernel was divided into four quarters, and attempts were made to pop these pieces. It was found that none of the quarters from that end of the kernels which had lain next the cob would pop, while some of the quarters from the outer end of the grain were popped successfully, although other specimens would not pop. It did not appear, however, that there was any difference, as to popping, according as the quarter kernels came from one or the other side of the pointed end of the grain. The difficulty seemed to be that so large a portion of the surface of these fragments (quarter kernels) was practically denuded that there was not skin enough left to help out the act of popping.

V. Several kernels of pop-corn were cut in halves, crosswise, the pieces were soaked in ether during five days and then dried for an hour or two at 100° C. On being heated, those halves which came from the outer, pointed end of the kernels popped successfully, while none of the halves from the inner end of the kernels would pop. They merely swelled a little.

VI. The skins were removed from some kernels of pop-corn which were then soaked in ether during three or, in some instances, four days, and subsequently dried at 100° C. No success was met with on trying to pop them. They behaved like the skinned kernels that had not been treated with ether.

It is plain, from the foregoing trials, that the skin of the grain exerts a very decided influence on the act of popping. It would appear, indeed, that both the structure of the individual starch grains in the kernel and the toughness of the restraining skin which envelops them all, act to control or modify the manner in which the moisture in the starch grains when suddenly heated is converted into steam of such high tension that the explosive act of popping results, whereby both the skin of the seed itself and the envelopes of most of the starch grains in the seed are ruptured.

No. 6.—*Observations on a Malt-Glucose, known as "Midzu-ame," made in Japan from Rice and Millet.* By F. H. Storer, Professor of Agricultural Chemistry in Harvard University, and George W. Rolfe, Instructor in Sugar Analysis at the Massachusetts Institute of Technology.

Several years ago one of us chanced to read in E. R. Skidmore's Jinrikisha Days in Japan,* the following statement:—

"Uraga (near Yokosuka) is notable as a place where *midzu-ame* or millet honey is made. The whole town is given up to the production of the amber sweet, and there are certain families whose midzu-ame has not varied in excellence for more than three hundred years. The rice or millet is soaked, steamed, mixed with warm water and barley malt, and left to stand a few hours, when a clear yellow liquid is drawn off and boiled down to a thick syrup or paste, or cooked until it can be moulded into hard balls. Unaffected by weather, it is the best of Japanese sweets, and in its semi-liquid state is twisted out on chop-sticks at all seasons of the year. The older and browner the midzu-ame is the better. It may be called the apotheosis of butter-scotch, a glorified oriental taffy, constantly urged upon one for one's own good, and conceded by foreign physicians in Japan to be of great value for dyspeptics and consumptives. Though prepared all over the Empire, this curative sweet is the specialty of Uraga; and the secrets and formulas held in the old families make for Uraga midzu-ame, as compared with other productions, a reputation akin to that of the Grande Chartreuse or Schloss Johannisberger, among other cordials or wines. Street artists mould midzu-ame paste, and blow it with a pipe into myriad fantastic shapes for their small patrons; while at the greatest banquets, and even on the Emperor's table, it appears in the fanciful flowers that decorate every feast."

It appeared from this statement that the Japanese have for several hundred years been accustomed to prepare a malt-glucose of superior quality which in respect to excellence had no counterpart in Europe. It was not until the year 1814 that the fact became known in Europe in any definite, scientific way that starch can be changed to a kind of sugar by the action of albuminoid matters in a state of change such as exist in malt prepared from barley or from the other cereal grains. Kirchoff announced this discovery at a meeting of the Academy of St. Petersburg in 1814,* and it is said that syrup prepared in accordance with his directions was soon afterward obtainable in Germany.† But this remark probably applied to products from the earlier process of Kirchoff in which starch was treated with sulphuric acid. The discovery of this process was published in 1812 and attracted immediate and widespread attention. Several years after Kirchoff, Dubrunfaut,‡ in France, published the results of his investigation of the matter and forecast the discovery (in 1833) of diastase, maltine, and the other enzyms with the remark "Hordein or some other substance in the barley first liquefies the potatoes (starch)." Since then much glucose has been made in this way in Europe to be sold as such, though the practice of the present time is most decidedly to prepare it by means of acids rather than by means of malt.

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* New York, 1891, page 37.

In view of the historical interest of the subject, we applied to a recent graduate of the Bussey Institution, Mr. Shin-ichi Takaki, of Tokio, to procure for us some of the material, and to learn what he could with regard to the details of the process of making it. We are under great obligations to Mr. Takaki for the effective manner in which he has complied with our wishes. A bottle of the material procured by him, labelled with Japanese words meaning "millet midzu-ame" was most distinctly a highly refined article, — such as an apothecary would be apt to call "an elegant preparation." It was a dense, clear syrup of light amber color, closely resembling the best commercial glucose of American manufacture in respect to its physical properties and taste, except that it has a faint though perceptible odor of malt. As contrasted with the "malt extracts" now so commonly made in this country for physicians use, the midzu-ame is incomparably superior both in appearance and in character. Its most prominent feature is its freedom from all foreign extractive matter, being in every way except the minor point of color up to the requirements of a first-class commercial glucose. It is non-crystallizable (colloidal), and is practically a pure carbohydrate, being what is known technically as a neutral sweet. Excepting the natural sweetness it is practically tasteless, there being only the slightest intimation of its malt origin. Like glucose it would undoubtedly take any flavoring desired, and it is safe to say that for the American taste, accustomed to less simple confections, some kind of flavor would be desired for by itself the taste of the midzu-ame, though sweet, is rather insipid. The sample contained about 0.23 per cent. of ash reckoned on the anhydrous substance.

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* See Schweigger's Journal für Chemie und Physik, 1815, **14.** 389, 394, 396, 398.

† Leŭchs, J. C., Die Zuker-Fabrikation, Nürnberg, 1835, page 30, *note.*

‡ Memoire sur la Saccharification des Fecules, Paris, 1823.

At first sight it seemed probable that the starch from which the midzu-ame was made must have been purified to a considerable extent rather than that the conversion had been carried out upon the crude grain itself. On being tested with the polariscope, its specific rotatory power was found to be $([a]_{\mathrm{D}386})$ 150°. Its cupric reducing power (K_{386}) was .432. These results correspond quite closely with the values given by O'Sullivan,* and by Brown and Heron,† for the transformation product made by treating starch with malt extract at a temperature below 63° C. for a few minutes and rapidly cooling. The constants given by O'Sullivan for the staple product thus formed are $[a]_{j386} = 170.6$ $([a]_{\mathrm{D}386} = 154)$ and $\mathrm{K}_{386} = .441$.

The composition of the anhydrous carbohydrate, calculating from the reducing value of .432 obtained by our analysis, is maltose 70.8 per cent. and dextrin 29.2 per cent., while a well made "commercial glucose" manufactured in this country from Indian corn, by way of acid hydrolysis, having a composition corresponding to a maximum content of maltose would contain 46 per cent. of maltose and about equal proportions (27 per cent.) of dextrose and dextrin.

It is of interest to note that, practically speaking, the midzu-ame contains very much the same proportion of dextrin as is contained in the American glucose above cited. But dextrin is a very important constituent of a syrup which is to remain permanently a syrup. In the making of glucose — as distinguished from "grape sugar" (starch sugar) — it is recognized as an essential requirement that enough dextrin should be left in the finished product to prevent any crystals of sugar from separating out when the syrup comes to be concentrated. Evidently, a solution of dextrin when present in due proportion has the power to hold dissolved both maltose and dextrose in such manner that neither of them shows any disposition to separate in the form of crystals. It is the non-crystallizable property of glucose that makes it specially valuable for the confectioner. Its power of "cutting the grain," as the technical term is, renders it much more useful for the candy-maker than "grape sugar" (starch sugar) could be. In fact, most of the "grape sugar" made in this country appears to be used not by the confectioners but by brewers for making sparkling ale.

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* Journal London Chemical Society, 1876, **14.** **(30).** 136.

† Journal London Chemical Society, 1879, **35.** 630.

Beside obtaining for us the sample of midzu-ame above described, Mr. Takaki reported that from what he could find in books and learn from letters written to him from Japan the process of making midzu-ame may be described as follows: —

"I. Take one measure of barley and soak it in cold water for 12 to 14 hours. When the barley has sprouted about three-tenths of an inch, take it from the water and dry it in direct sunlight for a day, then grind and sift it and preserve the powder or flour.

"II. Take a particular kind of rice that is especially glutinous and soak one measure of it in water during 12 hours in a rather warm place, then steam it in an iron kettle. Take out from the kettle the rice and the grating on which the rice had rested therein and throw away the water. Put back the rice directly into the kettle and stir it until it has become almost semi-liquid, then put in the powdered barley-malt and stir well. Pour in as much warm water as would amount to ten times the volume of the rice originally taken, cover the kettle, and leave it at rest for 12 hours. Then filter through a cloth bag, and boil down the filtrate until it becomes viscous.

"This is the way in which midzu-ame is made in south central Japan, where the climate is warm all the year round. I am told that climate, the quality of the water used, the stage of development of the sprouts on the barley, the degree of stirring, etc., affect the quality of the product more or less. Millet can be substituted for rice, the essential process is identical in both cases. Whether there is any further treatment or refining of the product I am not informed.

"We do not regard midzu-ame as food, in the same sense as we regard rice, fish or meat, but rather as a mere sweet. People eat it as they eat candy or cake, and it is thought to be an excellent means of stopping coughing.

"Midzu-ame and the liquor called *sake* are not made in the same establishments. While the making of midzu-ame is a comparatively small business, the brewing of sake is a great industry by itself that has been handed down from the legendary ages. It is interesting to note that sake and midzu-ame are both made from the same kind of rice (the glutinous variety). In sake-making "koji" is used, the substance from which Dr. Takamine extracted the enzym known as taka-diastase.

"The literal meaning of the words 'midzu-ame' is *water*-ame. 'Ame' is the name of a kind of candy — hard, sweet, white — made of midzu-ame: it is simply midzu-ame solidified. Water-ame or midzu-ame, therefore, is a name given in contrast to ame. In other words, midzu-ame is liquefied ame."

Thus far Mr. Takaki, who has also called our attention to the following statement taken from "A descriptive catalogue of the agricultural products, exhibited in the World's Columbian Exhibition": *—

"The manufacture of ame in Japan dates as far back as one hundred years before the Christian era. In early times when sugar was not yet known, ame was used for flavoring articles of food, and even at present it is sometimes used in place of sugar for cooking. Ame is made of malt and glutinous rice."

When our sample of midzu-ame first came to hand, we deemed it remarkable that a syrup so concentrated should be almost wholly free from color, since it is to be presumed that it had not been worked in a vacuum pan. But, on reflection, it seems probable enough that an exceptionally pure wort might be concentrated without injury even without the use of a vacuum pan, especially if it were faintly acid as it naturally would be from the presence of lactic acid, and we find evidence on record which supports this idea. Thus Ure* has said of one of his own experiments: "The extract of malt was evaporated to dryness at a temperature of about 250° F. without the slightest injury to its quality or any empyreumatic smell. . . . I found it impossible to make a solid extract from infusions of malt except at a much higher temperature than 175° F." And again† he says: "Well saccharified starch paste constitutes a syrup, poor, indeed, in sweetness when compared with cane syrup or that of the beet root, but then it does not spontaneously blacken into molasses by evaporation as solutions of ordinary sugar never fail to do when they are concentrated, even with great care. Hence the residuary syrups of saccharified fecula may all be worked up into a tolerably white granular mass which, being crushed, is used by grocers to mix with their dark-brown bastard sugars to improve their color." On page 779 Ure tells in detail what happens on evaporating hydrolyzed starch.

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* Agricultural Bureau, Department of Agriculture and Commerce.

On considering the history of a product so exceptionally pure and highly refined as is the midzu-ame, the thought naturally arises that in strict justice this substance should be compared not only with the European preparations of the present day, but with those of earlier periods, for it is evident that, after all, the midzu-ame is closely related to certain crude products that were made by our forefathers centuries ago and must have been contemporary with them in medieval times; products that is to say which had fallen so completely into disuse in Europe until quite recently that even the dictionaries of the present day no longer describe them as they really were in the beginning. Orgeat, — the very name of which is from the French *orge* (barley), — though said to be made from almonds nowadays, was originally a more or less concentrated barley wort. Ptisane, long a generic name for almost any mild medicinal drink, was barley-water, the *Gerstenwasser* of the Germans; while "barley sugar" — the *Sucre d'orge* of the French and *Gerstenzucker* of the Germans — is manifestly a mere modification of or improvement on the more or less highly concentrated barley wort that doubtless abounded in England in those early days when every family made its own ale at frequent intervals and put it to immediate use. Thorold Rogers,* in writing of medieval agriculture in England, has explained that "The largest part of the land under the plough was occupied by crops of wheat, barley, and oats. Wheat was the customary food of the people of this country from the earliest times. Even if the evidence were not abundant on this point, the breadth sown annually would be conclusive proof. Barley was sometimes mixed with wheat in the allowances made servants, but its chief use was in the manufacture of beer, which seems to have been continually brewed in small quantities and for immediate consumption."

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* In his Dictionary of Arts, Manufactures, and Mines, 4th Edition, Boston, 1853, **1.** 135.

† Ibid. **2.** 778.

It was at a later period, manifestly, when cane sugar came to be imported freely from abroad that the custom arose of adding some of the colonial product to the malt extract to increase its sweetness, and it was at that time doubtless that the name "barley-sugar" came in, for the word *sugar* had previously been unknown. It is in some sort by error that the dictionaries nowadays describe barley-sugar as "sugar boiled (formerly in a decoction of barley) till it becomes brittle and candied,"† for this statement fails to describe adequately the original article (sweet wort) and does not convey a just idea of its successors. During the larger part of the nineteenth century the product known, in New England at least, as *barley candy*, though doubtless a descendant of barley-sugar, was far enough from being a legitimate blood relation. In point of fact, this barley candy had not the least connection with barley, unless perhaps in respect to color, for it was simply cane sugar melted, together with a little water, until it had been converted to the glassy modification, while a small portion of it had been changed to caramel which imparted to the candy a tint of straw color. Sometimes traces of flavoring matters were added to make the candy more palatable. It is said, however, nowadays, that in the preparation of barley candy some 30 to 60 per cent. of glucose are habitually melted together with the cane sugar, so that, practically speaking, there has been an actual reversion to that early method of making barley-sugar where maltose and sucrose were commingled.

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* J. E. Thorold Rogers, History of Agriculture and Prices in England from 1259 to 1793, Oxford, 1866, **1.** 26, 27.

† Century Dictionary, New York, 1889, **1.** 454.

The sugary matters obtainable from unfermented barley wort were doubtless of some importance formerly in northern countries in times when cane sugar was unknown to the western nations, and honey, whey, and malt extract were the only available saccharine matters. According to Du Chaillu,* a dark brown preparation of milk-sugar, known as *myost*, is still made on Norwegian saeters by evaporating whey to dryness. It is eaten in thin slices, and with bread and butter. Women and children are especially fond of it.

These primitive products represent what may fairly be called the bronze age in respect to methods of sweetening, a stage in the progress towards civilization which the Japanese appear to have arrived at very early; that is to say, they seem to have systematized their methods of procedure long ago, and speedily to have brought them to perfection. The point of special interest is that at very early periods, hundreds of years ago, most nations learned how to obtain sweetness from the cereal grains by methods of changing the starch in these grains to sugar. As a means of obtaining alcohol from such sugar these methods have everywhere been largely developed and perfected, though as a source of sugar for household use they languished and fell into disuse in Europe, and were practically discarded altogether when cane sugar, brought from southern countries, had become abundant. But while the making of sugar in this way was given up long ago by the western nations, the Japanese developed and improved the old process and brought it to perfection some centuries apparently before it was taken up anew in Europe, for it is to be remembered that it is only so recently as the last half of the nineteenth century that any glucose was made in Europe that could for a moment be compared, in respect to excellence, with the normal Japanese product.

In view of the great differences between Japan and the United States as regards crops and agricultural and manufacturing methods and processes, it is not easy to form a just opinion as to the possibility of replacing economically the Japanese method of making midzu-ame, by means of malt, in many small establishments, by the American method of acting in large factories by way of acid hydrolysis. As a matter of course the present American method of procedure is better and cheaper in so far as concerns the United States, with its cheap and abundant crops of Indian corn; but rice and millet also are cheap grains that could doubtless readily be "converted" by the acid treatment, and maize is readily grown in eastern countries. Still, on taking everything into the account, it may well be true that the Japanese, in respect to glucose making, have already arrived at a point of supreme excellence as regards their own conditions and circumstances.

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* In his Land of the Midnight Sun, London, 1881, **1.** 395.

It may be remarked in passing of the glucose and "grape sugar" (starch sugar) that are prepared nowadays by thousands of tons in the United States, that they are for the most part products of a high degree of purity. Though sulphuric acid is still used in some factories, as the hydrolyzing agent, hydrochloric acid has replaced sulphuric in most establishments. When the conversion of the starch is completed the acid is neutralized with sodium carbonate so that only sodium chloride (common salt) is formed; the quantity of acid necessary for conversion is so small that the salt in the product may amount to no more than 0.2 to 0.25%, and the finished glucose may contain no more than 0.4% of total inorganic matter.

It is noticeable that our forebears, like the Japanese of the present time, flattered themselves that their barley sugars, ptisanes, and orgeats, possessed curative properties, and it must be admitted that there may have been some justice in the idea at a time when the dietaries in common use contained very little sugar. Many an invalid or feeble person may have profited by the occasional addition of palatable saccharine matter to food that was coarse and repugnant. Marchant† has called attention to the fact that "Medicated ales make a large article in the old English dispensatories" . . . "We meet in some dispensatories with syrup of ale made by boiling that liquor to a consistence; this is used against obstructions in the kidneys, etc., etc." But with the advent of cane sugar it became customary to attribute equal or even greater therapeutic virtues to this substance * — not to mention opposing views which have at times been intemperately expressed.

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* W. T. Marchant, "In Praise of Ale," London, 1888, page 44.

† Compare, for example, Benjamin Moseley, M.D., A Treatise on Sugar, London, 1800, *passim*.

Meanwhile, there are to-day many people who hold the supposed curative properties of malt extract in high esteem. Indeed, it is a very remarkable fact that the use of malt extracts has taken on a new lease of life in recent years both in Europe and, more particularly, perhaps, in this country, during the last twenty-five years. According to Fordred,* Mertens, in England, as early as 1863, prepared in vacuum pans a product called solid wort for exportation to the colonies, there to be made into beer. Coleman † tells of a liquid resembling British porter, and labelled "malt extract," that had been sent to England from Germany pretty freely during several years. But it appeared on analysis that this "extract" contained 4% of alcohol, and was to all intents and purposes a kind of beer. It is of a similar product that the United States Dispensatory ‡ remarks: "Sometimes a very strong beer has been put upon the market under the name "extract of malt."

Several different methods of making malt extracts have been suggested by apothecaries in this country,§ but the officinal article as described in the United States Dispensatory, 1899, pp. 1507, 1716, is prepared simply by macerating and digesting powdered malt with water at temperatures not exceeding 55° C., straining the mixture with strong expression and evaporating the strained liquor rapidly at a temperature not exceeding 55° C., either on a water bath or vacuum apparatus, to the consistence of thick honey. Improvements on this process consist in treating the malt with an alkaline solution to neutralize fatty acids, that might impart a bad taste to the product, and in pressing the extract in press-cloths to separate solid matters so completely that the extract shall be obtained as a clear liquid. The remark is made incidentally that a great deal of commercial extract of malt has been mixed with glucose "to a surprising extent."

According to the United States Dispensatory, 1899, page 1717, a dry extract of malt has come into extensive use as an infant's food. It is in the form of a straw-colored coarse powder, made by drying the thick syrupy extract mixed with starchy matter.

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* Journal of the Society of Arts, London, 1876, **24.** 185.

† London Chemical News, 1878, **37.** 177.

‡ Philadelphia, 1899, page 1716.

§ For one of them see Cooley's Cyclopædia of Practical Receipts, 7th edition, London, 1892, **2.** 1011.

It is administered dissolved in milk or in water. During the last fifteen or twenty years large quantities of malt extract have been made in this country on the manufacturing scale for the use of physicians and for compounding pharmaceutical preparations which are sold under various names and contain active medicaments of one kind or another beside the mere extract of malt.

We have met with one variety of malt extract that is sold as a "health food" under the name of malt-honey. It is a rather dark-colored, viscid syrup, less sweet than honey, and appears to be an extract of malt boiled down as such, and to contain unchanged the albuminoids and carbohydrates of the malt. Probably these constituents have purposely been left in the extract in order that the product may be more palatable and the better serve as a tolerably complete food, whereas the midzu ame has manifestly been made from a fairly pure starch upon which malt has been made to act in very small quantity and merely as a hydrolyzing agent. This so-called malt-honey on being tested as to its specific rotation and reducing power gave the following figures: $[a]_{D_{386}} = 138.7 = [a]j$ 153.4; K_{386}.465, Ash 0.8, water about 21%.

These figures, however, are merely approximate, and are not readily interpreted because of the presence of albuminoids, sugary and extractive matters, for which no corrections can be made.

As a question of folk lore or of archæology — not to say of the influence of governmental intermeddling — it would be a highly interesting study to determine, if possible, how it happened that the use of evaporated barley wort in domestic economy came to be so thoroughly superseded in western Europe when imported sugar had become comparatively cheap and abundant. It is not improbable, perhaps, that the prevalence of stringent excise laws relating to malt may have prevented everyone but the brewers from making this material. It is true at all events that, until the recent revival of malt extracts for medicinal use, it is not easy to find in books any explicit references to the use of worts as such. One trouble is that in early times it often happened that writers did not take pains to distinguish clearly between simple sweet extract of malt (wort) and the ale which results from the fermentation of the malt extract. Thus, the definition of wort in Johnson's dictionary is "new beer, either unfermented or in the act of fermentation. Bacon." The confusion due to this lack of definite description is similar to what might occur to-day in case an author in discussing cider should fail to specify whether he meant sweet cider or hard cider.

A writer in the journal "Every Saturday,"* regards it as "probable that the ale of our early ancestors — at least the great bulk of it — was intended to be drunk sweet and new." Another writer† assures us that ale, the favorite drink of our Saxon forefathers, was a thick, sweet, unhopped liquor; and there is no lack of testimony to the effect that a distinction was made originally in England between ale, which was a liquor brewed from malt, to be drunk fresh, and beer, a liquor brewed from malt and hops, intended to keep.‡ New ale and wort were often, or even usually, regarded as synonymous terms. The use of hops appears to have been not wholly unknown in England as early as 1440, though it was a century later that they came into general use.

It is perhaps not wholly impossible that a beverage known as Stepony, Stipony, or Stipone, that was procurable in England in the seventeenth century, may have been an unfermented sweet wort, though there seems to be no proof of this idea, and the descriptions of the substance itself are meagre and various. It is spoken of sometimes as "a kind of ale," and at other times as "not really ale," but "a sort of sweet liquor" . . . "drunk in some places in London in summer time." § Mr. T. H. B. Graham, writing in the Gentleman's Magazine ‖ on "The Malt Liquors of the Ancients" and "The Malt Liquors of the English," has given numerous references, some of which manifestly apply to the use of sweet wort rather than to beer proper.

Paulus, ¶ in a letter from Egypt, written in 1716, tells of a preparation of concentrated wort as eaten in that country: "Wheat, swollen with water during several days, is dried, ground, shaken into a pan of water, and finally boiled down to a certain consistence. A sort of comfit called *Nede* results, which is very palatable and sweet, although neither sugar nor honey have been added to it. The inhabitants are fond of it and often eat it."

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* London, 1866, **1**. 409.

† Notes and Queries, London, 1860, (2.) **10**. 335.

‡ See, for example, Notes and Queries, 1860, (2.) **10**. 334.

§ For some of the statements and opinions concerning Stepony that have been made at various times see Notes and Queries, London, 1880, (6.) **2**. 308, 334, 523; 1881, (6.) **3**. 97, 130; 1881, (6.) **4**. 155, 457.

‖ London, 1892 (N. S.) **48**. 415 and **49**. 52.

¶ In the fifth volume of his Reisen im Oriente, as cited by J. C. Leuchs in his Die Zuker-Fabrikation, Nürnberg, 1835, page 31.

A writer in Notes and Queries * has reported that "in Russia soldiers make use of quass loaves, which are made of oat- or rye-meal with ground malt and hops, made into cakes either with plain water or an infusion of hops. Sometimes the extract of malt is added, which is nothing more than sweet wort evaporated to the consistence of treacle. The cakes are then baked and kept for use. Infused for 24 to 30 hours in boiling water they make a wholesome, nourishing, and strengthening drink."

Just as evaporated barley wort formerly furnished a substitute for sugar at the north, so did evaporated grape juice in some southern countries. It has been said † that even in times of high antiquity the unfermented juice (must) of grapes was habitually boiled down in Syria to the consistence of honey, and was exported to Egypt in that form. It would appear, by the way, that formerly the term *must* was applied to wort from barley as well as to the juice of grapes. Tizard ‡ says that "in old dictionaries we find new ale called *mustum*, which is a general term for a drink not brought to maturity."

Parmentier § set forth long ago, in a voluminous "historical survey of saccharine matter," a multitude of instances in which, from the times of the Romans downward, evaporated grape juice has been prepared to be used as a substitute for honey. For that matter it is familiarly known that in many wine countries a practice has prevailed of boiling down a part of the juice expressed from the grapes in order that by adding this product to the remainder of the juice that is to be fermented, body and flavor may be given to wines that would otherwise have been thin and poor.

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* London, 1857, (2.) **4.** 290.

† By Shaw, as cited by Aikin in Transactions of the London Society of Arts, 1838, **51.** 158. See also Michaelis in Gilbert's Annalen der Physik, 1813, **44.** 65, *note*.

‡ W. L. Tizard, Theory and Practice of Brewing, London, 1846, page 249.

§ Annales de Chimie, 1811, **80.** pages 89, 293.

As is well known to chemists, Proust in Spain studied the question of making sugar from grape juice with the view of checking the waste of large quantities of grapes that could not be made into wine in that country. He obtained a coarse, solid sugar, but found that the cost of getting it was at that time larger than the cost of importing cane sugar from the colonies. During the Napoleonic wars the process was taken up seriously under Parmentier's * guidance, and put to practical use. For a time it was encouraged so strongly by the French government that considerable quantities of thick syrup thus prepared were actually disposed of in commerce.

After the foregoing article had been written out, as here printed, we noticed in the Index Catalogue of the Library of the Surgeon General's Office in Washington (volume 8, page 559, column 1), that a Japanese physician had published a paper on the same subject, in his native language. Through the courtesy of the library custodians at Washington and at Cambridge we have had opportunity to see the paper in question and to have it translated, as set forth on the next page.

Still later, after the types of this article had actually been set up, one of us happened to stumble upon a reference to a paper by Wiley and McElroy, entitled "Midzu Ame, or Japanese Glucose, which was read at a meeting of the Washington Chemical Society on January 14, 1892, and published in February, 1892, in the journal, now gone out of existence, called "Agricultural Science," volume **6**, No. 2, pp. 57–62. Had we known betimes of this work our own paper would have been written on somewhat different lines. We would have refrained, for example, from presenting the unnecessary repetition of the technical description of the Japanese processes of manufacture. But in the main our own point of view has been widely different from that of the authors now in question. The reader will have noticed that the chief purpose of our essay has been to call attention to a remarkable invention, which deserves to be classed in the list of oriental discoveries together with such items as the art of printing and the mariners compass, and particularly with chemical inventions, such as the manufacture of gunpowder and of sake, the testing of the precious metals, the making of alum and other saline products, and the intelligent use of indigo, madder, and other dye stuffs.

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* Annales de Chimie, 1811, **80**. 313, and 1813, **88**. 104.

We have sought to bring into view the historical relations that subsist between the making of midzu ame, which long ago became an article of economic importance in Japan, as the national sweet, so to say, and the malt products of our early European ancestors, which really represent glimmerings of industrial efforts directed towards the same end. It is not improbable that these efforts might eventually have resulted in success, if pursued on the original lines, had it not been for the fact that they were checked, or smothered, as it were, by the advent of cane sugar. Practically it happened that it is only in comparatively recent times that the making of glucose, whether by means of malt or of acids, has been brought by the western nations to the perfection attained long ago by the Japanese.

No. 7. — *Note on Ame.** By Yei Furukawa, M.D. *Translated from the original Japanese by Shin-ichi Takaki,* Bachelor of Agricultural Science.

Ame consists for the most part of maltose and dextrin. It is made with koji and rice that is well nigh free from nitrogen, and contains no more than traces of albuminoids, fat, and organic matters.

There are two kinds of ame : hard (kata ame) and soft (midzu ame). Ame is a substance of a light yellowish color, and it has a noticeable sweet flavor. All the carbohydrates contained in it are soluble, and in our country children and women are particularly fond of it. In my native province, Saga, it is customary to make presents of ame to women convalescing after childbirth; a practice which is highly proper from the hygienic point of view.

On the recent occasion of compiling a Japanese pharmacopœia it was reported that some sagacious person had suggested that Syrup † should be made from ame, but the idea was not accepted. The objection was made that patients might not be willing to regard as medicine anything made from a substance so familiarly known as ame is. I am not sure whether this story is true or not; but if it is true the refusal to use ame is regrettable, for it is an excellent substitute for syrup. The incident goes to show how shallow the knowledge of the populace still is. Even syrup would be liable to lose the credit in which it is now held if we were to explain to the common people that syrup is simply a concentrated solution of sugar. On this account, therefore, the action of Dr. Yogen Kusano is commendable, who — in following the formula of Dr. Bertz — has added from one-half to one per cent of ferrous lactate or ferric phosphate to ame, and has purposely called the preparations Ext. Malti Ferratum. It would be more proper to call them ferruginous ame. The name of the Ext. Malti Ferratum that is generally used in the hospital attached to the Imperial University at Tokio does not designate ame, but the extract of barley malt.

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* From Tokei Iji Shinshi (New Medical Journal of Tokio), No. 364, March 21, 1885, page 355.

† "Syrup" = (Sirupus Simplex). Dissolve 65 parts of sugar in 35 parts of distilled water. Specific gravity 1.32. — From "Japanese pharmacopœia."

There are more than thirty houses in Tokio where ame is made, and the annual product amounts to 280,000 Japanese pounds in this city. Besides this, the quantity given to patients as Ext. Malti Ferratum must surely be enormous.

I. *Manufacture of Ame.* — Ame is made from rice or from glutinous rice (*oryza glutinosa*) that is deficient in nitrogen;* rarely it is made from Indian corn or from sweet potatoes. The rice or the glutinous rice is softened by passing steam through it, and the starch of the rice is converted into sugar by adding water and malt made from barley. The proportions of these matters are as follows: —

Rice	15.00	kilogrammes.
Malt	2.36–3.54	"
Water (at 50°)	54.00	"

After thoroughly mixing these things in a vessel, the latter is covered with a straw mat to hinder it from cooling off and left to itself for about six hours. The product is then filtered through a bamboo basket. The filtrate is of excellent quality, and is called first rate ame. The residue is again filtered through a cloth bag, and an inferior article, known as second rate ame, is made from this filtrate.

Bleached ame is made by treating the foregoing filtrates with alum, which precipitates some impurities and coloring matters. After concentration finished ame will not change its composition, not even after several years. Ame that is three years old will retain the texture of vaseline though maltose by itself would crystallize.

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* From a work "On the manufacture of Sake," by K. Yagi, Graduate of the Tokio Agricultural College, it appears that there are three kinds of rice: upland rice (Okabo), ordinary rice (Uruchi), and glutinous rice (Mochigome).

Among the three the upland rice and the ordinary rice are the same in their chemical constituents; but the glutinous rice is different.

If we treat the flour of upland rice or ordinary rice with iodine solution, we recognize the presence of starch by the deep blue color produced. In the case of glutinous rice treated with the same solution we get a dark red color instead of deep blue, thus proving the non-existence of starch in it.

This substance different from starch has been called Amylo-dextrin. S-i. Takaki.

Stick ame is made from midzu ame by pulling it many times so that air is mixed into it. But if the stick ame is heated to drive out from it the enclosed air the original color and texture would reappear.

II. *Constituents of Ame.* — The composition of ame will appear from the following table: —

	Rice Ame.		Glutinous Rice Ame.	
	Moist.	Dry.	Moist.	Dry.
Water	17.40	——	15.05	——
Maltose	58.10	70.34	50.27	59.18
Dextrin	23.30	28.20	32.98	38.83
Protein	0.83	1.00	1.16	1.36
Oil	0.05	0.06	0.08	0.09
Ash	0.32	0.40	0.46	0.54

Since the rice ame is rich in maltose and poor in dextrin it has a sweeter flavor than the glutinous rice ame, the flavor of which is weaker in consonance with the smaller quantity of maltose and the larger amount of dextrin in it. Since the proportion of nitrogenous matter in both kinds of ame is minute, this constituent would not readily suffer decomposition even if the ame were to be kept for a long time.

III. *Value of Ame.* — Rice ame costs on the average 10 to 12 cents a pound (Japanese) and the glutinous rice ame 15 to 18 cents; therefore for one dollar we can get the following quantities. [One Japanese pound equals 0.6045 kilo.]

	Rice Ame.	Glutinous Rice Ame.
Quantity	5.000 kilograms.	3.000 kilograms.
Maltose	2.905 "	1.506 "
Dextrin	1.166 "	0.989 "
Ash	0.016 "	0.014 "

[One Japanese pound is equal to 1.33 pounds avoirdupois, and it is a matter of common report that the value of one Japanese cent is equal to that of half a cent of United States money; i. e., one dollar Japanese is equal to fifty cents of American money. It is to be presumed, furthermore, that the prices above given refer to the retail cost of small samples bought in Tokio shops. On these assumptions it would appear that the retail price of midzu ame made from ordinary rice does not differ materially from the retail price of glucose as bought in small quantities in America at the present time.]

No. 8. — *Experiments made to test the question whether mannite can be regarded in any large and general way as serving as reserve food in flowering plants.* By F. H. Storer, Professor of Agricultural Chemistry.

For more than one hundred years chemists have occasionally detected the presence of mannite in a variety of phanerogams, though the discovery that this substance is apt to be formed through the hydrogenation of sugars in several different kinds of fermentations — notably the viscous, the lactic, and the butyric fermentations — has in several instances cast doubts as to whether the observed mannite really existed, as such, in the living plants whose parts were subjected to chemical examination. Gayon and Dubourg* have described a special ferment which changes levulose to mannite; and Mazé and Perrier† have found that a ferment in sour wine has similar properties. In presence of levulose this ferment causes mannite to be formed by the action of hydrogen liberated from water. Laborde‡ also has shown that the ferments which cause wine to "turn" are analogous to the mannitic ferment of Gayon and Dubourg. On experimenting with pure cultures they found that the ferments which "turn" wine do also produce mannite from levulose. Roos § had previously argued that the occurrence of mannite in some wines depends primarily on the action of micro-organisms on sugars in the must, and not necessarily upon the existence of mannite in the fruit.

Gerhardt,‖ in his time, remarked: "Since the mannite found in plants is nothing more than a product of the metamorphosis of sugar, it may well be that at a certain epoch of vegetation no mannite would be found." In this point of view the well-nigh constant occurrence of mannite in adult mushrooms and other fungi, and the fact observed by Muntz * and by Bourquelot † that this mannite is a product of the transformation of sugar (trehalose) during the ripening of the mushrooms, or even during the process of drying fresh young mushrooms, suggests the enquiry whether mannite may not be formed habitually in some flowering plants at those stages of development when the vital processes within the plant most nearly resemble those habitual to the mushroom. It would be of interest, also, to learn whether mannite and mannan are apt to be found accompanying one the other in those plants or parts of plants which habitually contain mannan.

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* Cited in Chemisches Centralblatt, 1901, (2.) 648, 649.

† Annales de l'Institut Pasteur, 1903, **17.** 587; Abstract in Journal of the Chemical Society of London, 1903, **84**^{2}**.** 745.

‡ Comptes Rendus, 1898, **126.** 1223 and 1904, **138.** 228. Abstract in Journal of London Chemical Society, 1904, **86**^{1}**.** 278.

§ Journal de Pharmacie, 1893, (5.) **27.** 405.

‖ In his Traite de Chimie Organique, **2.** 573, *note*.

A Methodical Test for Mannite.

The first point to be attended to was manifestly the devising of a test for mannite which should be trustworthy and more readily applied than the old plan of obtaining actual crystals of mannite and studying their properties in comparison with those of pure mannite. The idea lay near at hand that it should be easy to convert any product supposed to be mannite into mannose by way of oxidation, and to precipitate this mannose in the characteristic form of mannose-hydrazone by means of phenylhydrazin.

On trial, it appeared at once that mannite may readily be changed to mannose by means of hydrogen dioxide acting in presence of a trace of ferrous sulphate, and that the mannose thus formed may easily be detected by means of phenylhydrazin. For example, an aqueous solution that contained 0.6 grm. of mannite was treated with a small quantity of ferrous sulphate and 10 cc. of commercial hydrogen dioxide (the commercial 3% solution of the United States Pharmacopœia) and left at rest for half an hour. The mixture was then made faintly alkaline with sodium carbonate and filtered. The filtrate, acidulated with acetic acid, was evaporated to soft dryness, the residue was taken up with 1 cc. of water, and the solution was filtered. To the dark-colored filtrate 16 drops of a solution of phenylhydrazin acetate were added, and in the course of five minutes balls of mannose hydrazone appeared. Under the microscope many of these balls were seen to be beset with the blunt spines which are characteristic of this substance; on recrystalling the balls from diluted alcohol (3 parts alcohol of 95% and 1 part water) clear cut crystals and plates of mannose hydrazone were obtained.

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* Comptes Rendus, **76.** 649; 1874, **79.** 1182; and Annales de Chimie et de Physique, (5.) **8.** 60.

† Comptes Rendus, **108.** 568; 1890, **111.** 534, 578, and Journal de Pharmacie, 1893, (5.) **27.** 113.

It is worthy of remark that less favorable results were obtained in experiments where potassium dichromate was used instead of the hydrogen dioxide to oxidize mannite. It was made evident that the dichromate is less convenient than the dioxide for this purpose, though it might be made to serve fairly well in case the dioxide was not to be had. For example, a solution containing 0.2 grm. of mannite was treated with 1 cc. of dilute sulphuric acid and 20 drops of a 1:25 solution of potassium dichromate and the mixture was boiled for 1 minute. When cold it was nearly, but not quite, neutralized with sodium hydroxide, and sulphuretted hydrogen was passed to saturation to reduce the excess of chromic acid. The liquid was made faintly alkaline, filtered, acidulated slightly with sulphuric acid, and evaporated to soft dryness. The residue was taken up with 1 c.c. of water, the solution was filtered, and 16 drops of the phenylhydrazin acetate solution were added to it. Next day balls of mannose hydrazone, as well as osazone crystals, were plainly visible. Similar results were obtained in another trial where no sulphuretted hydrogen was applied to reduce the excess of chromic acid.

Precipitation of Mannite by means of Ammoniacal Copper Sulphate.

Experiments were made also to test the question whether the method of precipitating mannite with ammoniacal copper sulphate, as proposed by Guignet,* is delicate enough for the purposes of the present enquiry. As will be seen below, this method has considerable merit, though a better method is still to be desired.

To prepare the ammoniacal compound, crystallized copper sulphate in fine powder that has been dried for several hours at 100° C. is added little by little to strong ammonia. As soon as a deep blue precipitate begins to separate from the dark blue liquor the whole is boiled for a few minutes and is then allowed to cool. Fine blue needles separate, from which the mother liquor is removed by way of decantation. The crystals are washed, by decantation, with a small quantity of cold water, and dissolved in cold water to a deep blue solution, the fitness of which for precipitating mannite was tested as follows: One tenth of a gram of pure mannite, dissolved in 5 c.c. of water, was treated with a small quantity of the ammoniacal copper solution, the light blue precipitate was collected upon a filter, washed with cold water, and then suspended in warm water through which a current of sulphuretted hydrogen was passed. The black colloidal precipitate of copper sulphide was not readily filtered until after it had been boiled to make it cohere. The filtrate from the copper sulphide was evaporated to a small bulk and left to cool slowly in a watch glass. After standing over night crystals of mannite were plainly visible. On being weighed they were found to amount to 14% of the mannite taken. In a duplicate trial the mannite crystals were treated with ferrous sulphate and hydrogen dioxide, and finally with phenylhydrazin acetate. Mannose-hydrazone balls were obtained.

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* Comptes Rendus, 1889, **109**. 528.

Efforts that were made to treat the mannite-copper precipitate directly with hydrogen dioxide, that is to say, to omit the use of sulphuretted hydrogen altogether, failed. Thus, when 0.1 gram of mannite in aqueous solution was treated with the ammoniacal copper sulphate, and the washed precipitate, suspended in water, was mixed directly with a little ferrous sulphate and some hydrogen dioxide, and subsequently treated in the usual way, no indications of mannose hydrazone were obtained. In another trial a solution that contained 0.4 grm. of mannite was treated with the ammoniacal copper sulphate, and the washed precipitate was dissolved in a small quantity of diluted hydrochloric acid (1:4) before adding the ferrous sulphate and hydrogen dioxide. No mannose hydrazone was obtained, and no better results were got in a third trial, where the mannite-copper precipitate was dissolved in dilute sulphuric acid before applying the hydrogen dioxide.

Method of Percolation.

In order to avoid the risk that some mannite might be formed by way of fermentation when plants supposed to contain it are leached with water, it was deemed advisable to percolate the matters under examination with water maintained constantly at the boiling temperature. In practice, 100 grams of the powdered material to be tested are packed in a percolator a foot long and nearly two inches in diameter, made of tin ware. The lower part of this cylinder is constricted to an opening three quarters of an inch in diameter into which a glass tube is fitted by means of a perforated cork. In order to keep the percolator hot it is placed in a tin can six inches in diameter, filled with boiling water. The glass tube, that serves as the shank of the percolator, passes through a hole in the bottom of the can and is fastened there with a short piece of rubber tubing acting as a perforated cork. Into the water of the can, which acts as a jacket to the percolator, steam is blown constantly to maintain it at the temperature of boiling. Repeated small quantities of boiling water are poured in upon the matter in the percolator until some 200 to 400, or even 500, c.c. of percolate have been collected. Care is taken to add the water little by little, so that each new portion of it may "displace" the solution formed by the preceding portion of water.

To test the delicacy of this process of percolation when used in conjunction with the foregoing method of precipitating with ammoniacal copper sulphate, three grams of pure mannite were dissolved in water, and a sufficient number of small pieces of filter paper to absorb the whole of the liquid were soaked in this solution. The paper thus charged with mannite was placed in the percolator and 200 c.c. of hot water were made to pass through it little by little. The percolate was evaporated to one half its original volume, and mixed, while still warm, with a quantity of the ammoniated copper solution. The light blue precipitate was collected on a filter, washed with cold water, and then suspended in warm water through which sulphuretted hydrogen was passed to saturation, as has been explained already. In this case the black colloidal precipitate of copper sulphide was made to settle by adding four or five drops of a dilute solution of barium chloride and boiling. The filtrate, evaporated to a small volume over a water bath, was finally left to dry out spontaneously in a warm place. Needle-like crystals of mannite separated, and were found to weigh 0.293 grm.; i. e., there was recovered somewhat less than ten per cent of the mannite taken. On being dissolved in water, treated with ferrous sulphate and hydrogen dioxide, and afterwards with phenylhydrazin acetate, with the usual precautions, these mannite crystals gave, beside osazones, characteristic balls of mannose-hydrazone, which, on being recrystallized from 3 : 1 alcohol, gave excellent crystals and plates of mannose-hydrazone.

Sometimes, as in the case of fleshy roots, like the turnip, percolation is impracticable because the soft flesh is impermeable to water. In this event the material under examination was washed by way of decantation, using repeated small quantities of boiling water.

Tests for Mannite in Various Grasses.

As an example of the practical working of the process of percolation the following record of an experiment on oat hay may be cited. This hay was cut at the Bussey farm at the end of June, 1902, when the grain was "in the milk," and was stored in a dry barn until November, 1903. It was cut into small pieces and ground in a drug-mill to a fine meal. 100 grm. of the powder, packed in the percolator, were leached with boiling water until some 150 to 200 c.c. of percolate were obtained. After evaporation to a volume of 100 c.c., some of the ammoniacal copper sulphate was added. The concentrated percolate was so dark-colored that it was not easy to see whether any precipitate fell; but, on filtering, a small quantity of a light green precipitate was collected. After washing with cold water this precipitate was decomposed with sulphuretted hydrogen, and the filtrate from the copper sulphide was evaporated to a small bulk and allowed to cool slowly. A yellowish residue was obtained, which under the microscope showed crystals of mannite. When tested with ferrous sulphate and hydrogen dioxide, followed by phenylhydrazin, large balls of mannose-hydrazone were obtained, which yielded fairly good crystals and plates of this substance on being recrystallized from 3 : 1 alcohol. It should here be said that in an earlier experiment with the oat hay, where basic lead acetate, bone black, and alcohol were employed, no satisfactory evidence of the presence of mannite was obtained.

In the case of a sample of June grass (*Poa pratensis*) hay mown in June, 1903, and stored in a dry barn until November, both crystals of mannite and thereafter mannose hydrazone crystals were obtained, as they had been from the oat hay, by the method of percolation, precipitation with ammoniacal copper sulphate, and oxidation with hydrogen dioxide. Another positive result was obtained on examining fresh leaves plucked from the stems of bottle grass (*Setaria viridis*) early in September, 1903. The grass had flowered and some seeds had begun to ripen. Neither the stems nor the seeds were examined, but only the lower leaves. In this case 60 grm. of the fresh leaves cut into small pieces were extracted with 250 cc. of boiling alcohol of 75% ; the alcohol was driven off and the residue after having been washed with alcohol of 95% and with ether was taken up with repeated portions of boiling alcohol of 85%. In this way small successive crops of crystals of mannite were obtained which when treated with ferrous sulphate and hydrogen dioxide and subsequently with phenylhydrazin acetate gave crystals of mannose hydrazone.

An equal quantity (60 grm.) of the fresh leaves of the bottle grass was allowed to dry slowly during a fortnight on a shelf not exposed to the sun's rays, care being taken to turn the leaves once a day. The dry leaves were leached with alcohol as the fresh leaves had been leached and mannite was obtained from them apparently in somewhat larger quantity than had been got from the fresh leaves. Mannose hydrazone crystals also were obtained in due course. This method of extracting with alcohol is inconvenient because the mannite crystals are contaminated with much gummy matter.

It needs to be said emphatically that the foregoing three positive results, viz., the finding of mannite in oat hay, in June grass hay, and in the leaves of maturing bottle grass, differ decidedly from the results of the examination of various grasses collected at earlier stages of development for the most part, while growing in the fields. Thus, no mannite was detected in the samples of grasses mentioned in the following list: —

Fresh leaves of June grass collected on September 26, 1903.

Leaves of the June grass of September 26, that had been allowed to dry slowly in the shade.

Fresh leaves, each about 4 inches long, of tender young June grass collected on May 2, 1904.

Leaves of the June grass of May 2, that were allowed to dry slowly until May 26.

Fresh leaves and stalks of June grass collected on May 24 when the grass was 6 inches high and not yet in flower though buds had formed.

Leaves and stalks of the June grass of May 24 that were allowed to dry slowly until June 15.

Fresh June grass collected on June 10, at a moment when flowers were about to form. The leaves were 9 inches long and the stalks 18 inches.

Leaves and stalks of the June grass of June 10, that were allowed to dry slowly during a fortnight.

Fresh flower stalks and blossoms of sheep's fescue grass (*Festuca ovina)* collected on June 20, 1904. The grass was nearly in full bloom and the flower stalks were about 12 inches high. On being tested with Fehling's liquor, the percolate from this grass gave a strong reducing reaction, which went to show the presence of an appreciable quantity of sugar rather than of mannite.

Rowen hay (June grass) that had been mown early in September, 1903, and allowed to dry for a month on a table.

Leaves of timothy grass (*Phleum pratense*) plucked from the stalks of dead-ripe standing grass on July 22, 1904.

The negative results obtained in so many of the tests with grasses could not fail to suggest qualms of doubt as to whether the mannite-copper precipitate suspended in water is always decomposed by sulphuretted hydrogen so easily and completely as has been assumed. In order to test this point yet again a small quantity of pure mannite (0.25 grm.) was purposely mixed with a new portion of one of the specimens of June grass such as had given negative results as regards mannite when tested by itself in the normal way, and the processes of percolation, precipitation, etc., were carried forward in the sophisticated sample in the usual way with the result that the mannite that had been introduced was detected without any difficulty both in the form of crystals of mannite and subsequently as crystals of mannose-hydrazone. The grass employed in this trial consisted of the leaves and stalks of June grass collected on June 10, 1904, that had been allowed to dry slowly for a fortnight, as mentioned above.

It may be remarked that the motive for paying so much attention to the testing of grasses depended on the hope that some light might perhaps be thrown on the chemical changes which occur in the obscure process known to practical men as "sweating" which is manifested even in well-cured hay after it has been stored in the mow. It may still be true that mannite will be found more commonly in hay taken from barns than in grass plucked in the field. The results obtained with oat hay and June grass hay as above set forth tend to support this idea. Much labor would have to be expended if this question were thoroughly studied. I had fancied also that the conflicting statements of chemists as to the presence or absence of sugar in grasses might perhaps be composed if it could be ascertained at what times and stages of development some part of the sugars in young grass might change to mannite.

Old and New Oat Grain Contrasted.

It is not alone as regards new and old hay that practical men note a marked difference in respect to physiological properties which must depend on differences in chemical composition. These differences are exaggerated in oat grain according as it is new or old. It is a well-known fact that oats which have recently been harvested have a laxative and "softening" effect on horses. They are regarded as quite unfit to be given to animals that have to perform hard work. It is only after oats have been kept for some time in store that they become really fit for use.

In the hope of throwing some light on this matter, "old oats" of the crop of 1903, bought in August, 1904, were tested for mannite in contrast with "new oats" of the crop of 1904, bought early in September, 1904. But no mannite was detected in either of these samples.

200 grm. of each of the specimens were weighed out and ground to coarse meal. A percolator full of the old oats amounting to 150 grm. was percolated with hot water without much trouble though the operation was slow, while the remaining 50 grm. were leached by way of decantation with small successive portions of boiling water.

The meal from the new oats, on the contrary, formed a thick pasty mass on being treated with hot water and could not be percolated. Hence the whole 200 grm. were leached with successive portions of hot water. In both cases, the solutions were filtered and evaporated to a volume of almost 100 cc. before being treated with the ammoniacal copper sulphate, which seemed to the eye to give a somewhat larger (bluish) precipitate in the case of the new oats than was the (greenish) precipitate obtained from the old oats. But on treating these precipitates in the usual way no indications of the presence of mannite could be detected in either of them.

Tests for Mannite in Clover and Vegetables.

Clover. — No mannite was detected in leaves of red clover collected on September 28, 1903, neither in the fresh leaves nor in those dried slowly in the shade; nor was any mannite found in the fresh leaves, flower stalks, or blossoms of red clover collected on June 23, 1904, when the plants were passing out of bloom. The several parts of the plants were examined separately.

Onions. — No mannite was detected in new onions bought on September 15, 1904. After removal of the outer brown skins, the flesh of the onions was chopped fine and 300 grm. of it were subjected to hot water percolation and subsequent treatment in the usual way.

Cabbage. — No mannite was detected in a head of cabbage bought on March 11, 1904. The ground up cabbage, mixed with sand, clogged the percolator and had to be extracted by way of decantation, using repeated small quantities of boiling water and filtering through a tuft of cotton finally with the help of a filter pump.

Carrots. — No mannite was detected in the flesh of a yellow carrot bought on April 7, 1904. The material could not be percolated. It was extracted with boiling water, by way of decantation.

Parsnips. — No mannite was detected in the flesh of a parsnip bought on March 5, 1904. The material clogged the percolator and had to be extracted by way of decantation.

Turnips. — In the flesh of a yellow turnip bought March 17, 1904, mannite was readily detected. The material could not be percolated but was extracted with boiling water. After treatment with ammoniacal copper sulphate in the usual way, and thereafter with ferrous sulphate and hydrogen dioxide, and finally with phenylhydrazin acetate, large balls of mannose-hydrazone were obtained which gave good crystals on being recrystallized from 3 : 1 alcohol.

Celery Leaves. — No mannite was detected in the green leaves cut from the tips of celery stalks bought on November 21, 1903, January 14, 1904, and March 8, 1904. The three samples were dried and ground up together to a fine meal, which had to be leached by way of decantation.

Carrot Leaves. — Leaves of the white Belgian carrot were gathered on October 19, 1903, at the Bussey farm and allowed to dry until January 7, 1904. The powdered leaves were percolated with boiling water and the solution was precipitated with ammoniacal copper sulphate, in the usual way. A small quantity of crystals that appeared to be mannite were obtained on evaporating the filtrate from the copper sulphide, and on being treated with ferrous sulphate and hydrogen dioxide and with phenylhydrazin acetate they gave mannose-hydrazone balls. It was evident that the dried carrot leaves contained some mannite.

Tests for Mannite in Woods.

White Pine Wood. — Two attempts were made to find mannite in 100 grm. portions of the wood of a white pine tree collected at Hingham, Mass., in August, 1903, and one of the trials indicated that small quantities of mannite might possibly be present. That is to say in the trial where 100 grm. of the wood were percolated, a few crystals resembling those of mannite appeared in the filtrate from the copper sulphide, and on treating these crystals with ferrous sulphate and hydrogen dioxide and subsequently with phenylhydrazin acetate, a small quantity of balls and crystals resembling those of mannose-hydrazone were obtained. In the other trial where the wood was percolated with hot alcohol of 75% only a negative result was obtained. Though the results of the first trial were by no means wholly satisfactory they went to show the probability of mannite in the pine wood. Yet another trial made with pine wood collected in the spring, viz., on April 9, 1903, and percolated with hot water gave no indications of mannite.

Gray Birch Wood. — No mannite was detected in a 100 grm. sample of the wood of a gray birch tree (*Betula populifolia*) collected in October, 1895.

Lilac Wood. — In view of the fact that several chemists have reported the presence of mannite in young twigs and leaves of the common lilac (*Syringa vulgaris*) samples of these materials were subjected repeatedly to the method of testing which has been described above. Thus 60 grm. of dried leaves of the purple lilac plucked on May 7, 1903, were percolated and examined in January, 1904, but no mannite was detected.

100 grm. of lilac wood (free from bark) collected on May 7, 1903, and examined in February, 1904, gave no reactions for mannite.

Twigs $\frac{3}{16}$ to $\frac{1}{2}$ inch in diameter cut from a purple lilac bush on April 11, 1904, were freed from bark and were percolated and treated in the usual way. No mannite was detected.

Fresh young half-formed leaves of a purple lilac collected on May 10, 1904, gave finally some not particularly well formed crystals which may have been mannose-hydrazone though the quantity obtained was not large and the result was not really satisfactory.

Fresh young lilac shoots that had begun to be formed from buds on the twigs were collected on April 11, and allowed to dry. The powdered material was percolated and treated as usual. Some crystals that separated on evaporating the filtrate from the copper sulphide appeared to be *dulcite* rather than mannite and suggested the thought that previous observers may perhaps really have had to do with dulcite rather than mannite in their experiments on the lilac. None the less, in the final test with phenylhydrazin acetate, a few crystals were obtained which may possibly have been mannose-hydrazone, though the evidence was not conclusive.

Tests for Mannite in Fruits.

Dried Figs of commerce bought in Boston, July 7, 1904, were cut into small pieces which were ground in a mortar together with clean sharp sand to a friable mixture fit to be percolated with hot water. On allowing the percolate to stand over night at the ordinary temperature of the air, it solidified to a jelly which liquefied however on the addition of a small quantity of water and warming the mixture. The solution was now readily filtered and it remained limpid on cooling, but to our surprise no precipitate formed on adding some of the ammoniacal copper sulphate and none appeared on standing, not even when the mixture was left at rest until the next day. In the belief that the matter which had previously gelatinized might perhaps act to hold the mannite-copper compound in solution, basic acetate of lead was added to a small portion of the fig liquor (to which some drops of the ammoniacal copper sulphate had already been added), and the copious flocculent precipitate of lead oxide combined with organic matter was removed by filtration. On adding to the clear filtrate a new quantity of the ammoniacal copper sulphate a blue precipitate fell at once which was decomposed by sulphuretted hydrogen in the usual way. On evaporating the filtrate from the copper sulphide fine needle-like crystals were obtained which gave mannose-hydrazone balls and some excellent crystals of mannose-hydrazone, on being treated with ferrous sulphate, hydrogen dioxide and phenylhydrazin acetate. A similar result was obtained when the remainder of the fig liquor was mixed with basic lead acetate and the clear filtrate was thereafter treated with the ammoniacal copper sulphate, etc. It was evident enough that the figs contained mannite though perhaps in no very large proportion.

To see if any of the mannite-copper compound had been dragged down with the precipitate produced by the basic lead acetate, a quantity of this heavy precipitate was suspended in water and decomposed with sulphuretted hydrogen. After removal of the lead sulphide, the filtrate was evaporated as far as possible and the viscous residue was left in a cool place for several days. No crystals of mannite appeared and no reaction for this substance was obtained on dissolving the residue in water and treating it with ferrous sulphate, hydrogen dioxide, and phenylhydrazin acetate.

Cherries. — Large white cherries, firm and sound, that had grown in the vicinity of Boston, were bought on July 14, 1904. After removing the stems and stones, the flesh of the fruit was cut into small pieces and rubbed in a mortar to a pulp. The juice expressed by the rubbing was filtered and added in due course to the percolate obtained by heating some 300 grm. of the pulp with hot water. After evaporating the solution it was found to be somewhat acid and it was almost but not quite neutralized with sodium carbonate. On adding ammoniacal copper sulphate to a part of this solution no precipitate fell, not even when the mixture was left to stand over night. Basic lead acetate was added to the remainder of the solution, the precipitate that formed was removed by filtration and the excess of lead was thrown down by means of sulphuretted hydrogen. On adding ammoniacal copper sulphate to the clear filtrate from the lead sulphide no precipitate fell; hence the inference that the cherries contained no mannite.

Prunes. — Bought in Boston, July 18, 1904. Some 250 grm. of the prune flesh were rubbed with sand in a mortar to a friable mass. Since this mixture could not be percolated it was leached by treatment with many successive portions of hot water. Ammoniacal copper sulphate gave no precipitate on being added to a portion of the evaporated solution and none was obtained in another portion which had been treated with basic lead acetate, as above described. Hence the conclusion that the prunes did not contain mannite.

Raisins. — Bought in Boston, July 21, 1904. After the removal of stems and seeds, the flesh of the raisins was ground up with sand in a mortar to a mass that was readily percolated. In the percolate, concentrated by evaporation, ammoniacal copper sulphate produced no precipitate, and none was obtained in another portion of the percolate which was treated with basic lead acetate as described above. The absence of mannite was admitted.

Bananas. — The flesh of yellow bananas bought in Boston on July 27, 1904, was rubbed with sand in a mortar to a thick paste, part of which was percolated and another part leached by way of decantation with many successive portions of hot water. The evaporated and filtered percolates gave no precipitate with ammoniacal copper sulphate and none was obtained after treatment with basic lead acetate. The absence of mannite was admitted.

Apples. — No mannite was detected in Gravenstein apples bought September 20, 1904. After the skins and cores of the apples had been removed, 300 grm. of the finely chopped flesh were subjected to hot water percolation. The evaporated slightly acid percolate was nearly neutralized with sodium carbonate before adding the ammoniacal copper sulphate. The liquor was so dark-colored that no precipitate could be seen in it, and no precipitate was visible on the paper of the filter through which the liquor was passed. Sulphuretted hydrogen was now passed through the liquor to throw down the copper and, after the removal of the copper sulphide and the excess of sulphuretted hydrogen, basic lead acetate was added. The precipitate thus formed was removed by filtration and sulphuretted hydrogen was used to remove the excess of lead. After the removal of lead sulphide and the excess of sulphuretted hydrogen, ammoniacal copper sulphate was added and the light blue precipitate obtained was treated for mannite in the usual way but none was found.

The non-appearance of any precipitate on adding ammoniacal copper sulphate to the evaporated percolates from figs, prunes, raisins, bananas, and apples was noteworthy because precipitates had been obtained on adding the copper solution to the suspected solutions in all the previous experiments with grasses and vegetables, though in most cases, as has been shown, the precipitates were not due to the presence of mannite.

It should be added, that in several instances attempts were made to find mannite in grasses and clover by using basic lead acetate to remove from the percolates (either aqueous or alcoholic) coloring matters and other substances likely to cause interferences. The idea was to get rid of the excess of lead by means of sulphuretted hydrogen, to evaporate the filtrate from the lead sulphide to a small bulk and to leach the syrupy residues with hot alcohol which should take up the mannite. As a general rule, no success attended any of these trials, and excepting the case of the fig liquor, where a masking substance had to be removed, there was little encouragement to use either the lead acetate or alcohol.

My best thanks are due to my assistant, Mr. F. T. Dillingham, for his skilful coöperation in the conduct of this research.

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No. 9. — *A Field Experiment to Test the merits of Phosphate, Potash, and Nitrogen on a Fine Sandy Loam of peculiar character.* By Edmund Hersey, Instructor in Farming.

Nearly twenty years ago, at a time when few farmers in this vicinity had any just appreciation of the power of certain fine, sandy soils to bear crops, I had occasion to make some experiments on such land the results of which were so distinctly instructive that even at this late day they seem to be worthy of being put upon permanent record.

The field operated upon was at the top of a flat knoll some thirty feet above the surface of a small pond which lies near at hand. The soil of this field is a very fine, sandy loam slightly colored at the surface from the admixture of a small proportion of humus, but it consists principally of an extremely fine siliceous sand, well nigh free from flakes of mica, which reaches to an unknown depth. A well driven through this land came to the water-table at a depth of sixteen feet. Naturally enough, this sand readily drinks in and absorbs rain-water that falls upon it, and it is at the same time highly capillary because of its extreme fineness. It lifts water from below so easily and constantly that at times when neighboring farmers are complaining of drought it can be seen on scratching the surface of the soil in my field that there is plenty of moisture contained in it. Evidently the conditions are highly favorable for processes of nitrification, and as the results of the experiment showed they are well nigh ideal for the utilization of bone meal.

The field had long lain unused, excepting as a poor cow-pasture. It had probably not been ploughed for one hundred years, if ever, until shortly before my experiment. It was free from trees and bushes. On first trying to bring the land under cultivation, I was seriously misled by the advice of an acquaintance living on a farm one hundred miles distant from mine who had found that his soil needed potash more than any other fertilizer. After following his advice for three years and becoming entirely dissatisfied with the results obtained, which showed clearly enough that potash salts were not specially needed on my land, I determined to try a methodical experiment which should demonstrate what kinds of fertilizers were really called for, and determine once for all whether a potassic or a phosphatic or a nitrogenous fertilizer was specially needed to supplement the natural strength of the land. The crop employed in making these tests was Indian corn of the long-eared, eight-rowed yellow variety, and it was planted on strips of land in such manner that the plants were three and a half feet distant from one another in each direction. Between each plot boards over twelve inches in width were sunk in the ground to keep each kind of fertilizer in place.

After the corn had matured and been harvested, it was kept well into the winter in an airy place until it had become dry before it was shelled and weighed.

In the tables on pages 116 and 117 are given the kinds and money values of fertilizers that were applied to the several plots, and the bushels and pounds of shelled corn that were harvested from each plot; it being noted that the bushel of Indian corn weighs fifty-six pounds.

For the sake of easy comparison a rough approximation to the figures of the tables is given in the form of the black lines printed on pages 118 and 119. The best crop is represented by the longest line and the other crops follow in the shorter lines in the order of their merit, very nearly.

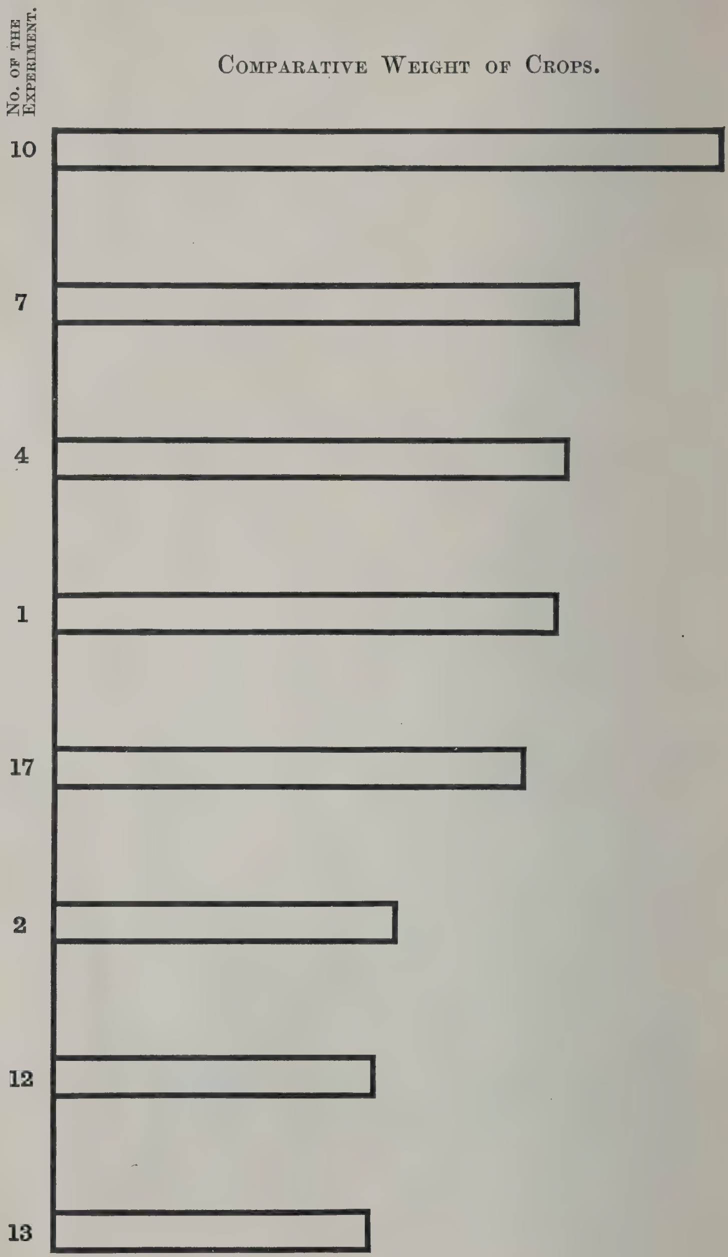
The marked superiority of the ground bone in all these trials was most unexpected, and is really not a little remarkable. In fact, the results of the experiment indicated so clearly the real needs of my land that I proceeded at once to set up a small establishment for "rendering" bones and for grinding the steamed bones to a fine powder. I have derived no small profit from the use of this fertilizer, and have had no little satisfaction in supplying myself and a few friends with an absolutely pure and unsophisticated article.

During eight or nine years after the conclusion of the experiment I applied the ground bone, at the rate of nearly one ton to the acre, with marked success, to a variety of garden crops, notably to asparagus and rhubarb, being led to do so by the knowledge that my previous treatment of the land must have surcharged it with potash, to say nothing of the store of potash naturally contained in the soil. But after this time I have occasionally applied small quantities of potash to keep up such supply as I have thought to be necessary for the best interests of the land, though these additions have been made for the sake of security as it were and from a desire not to scourge or force the land unduly rather than from any actual necessity. Meanwhile, the sandy soil has been kept singularly free from weeds, with very little labor.

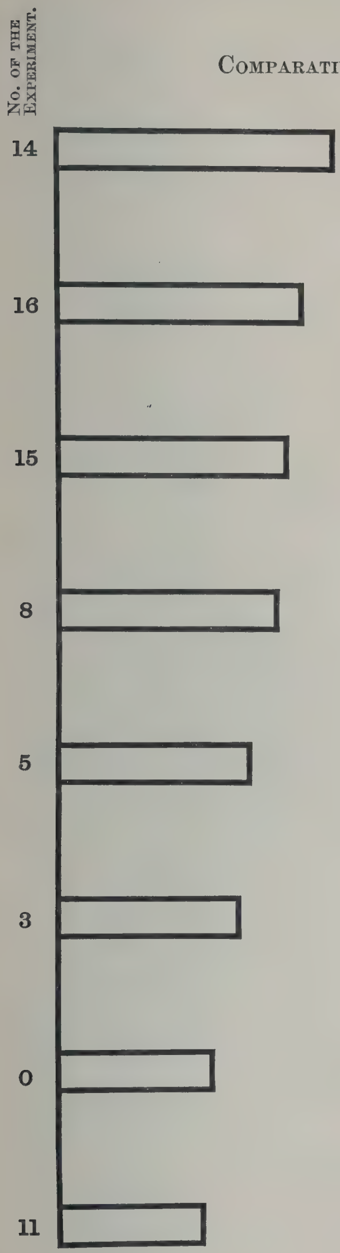
So very convincing were the results of this experiment that I have been accustomed to discuss it with my students as a practical illustration of the necessity that each farmer should inform himself by actual trial as to what his land needs in order that crops may be grown upon it to the best advantage. He should ascertain which of the three prominent elements of plant food predominates in his land, and which of them is not present in adequate supply for the crops he wishes to grow. Once well informed as to these points, he will be prepared to furnish what is lacking in his soil and will not be obliged to seek outside assistance or be perturbed by the conflicting statements of would-be advisers who may really have no better information or so good as what he might himself guess.

No. of the Plot.	Kind and Value of the Fertilizer.	Produced Shelled Corn to the Acre.	
		Bushels.	Pounds.
1.	Stable Manure, $30 worth to the acre (at $5 the cord)	41	26
2.	Nitrate of Soda, $30 worth to the acre	27	26
3.	Muriate of Potash, $30 worth to the acre	14	28
0.	No Manure	12	15
4.	Ground Bone (steamed), $30 worth to the acre	42	23
5.	Nitrate of Soda, $60 worth to the acre	15	35
6.	Muriate of Potash, $60 worth to the acre	00	00
7.	Ground Bone (steamed), $60 worth to the acre	43	00
8.	Nitrate of Soda, $120 worth to the acre	17	48
9.	Muriate of Potash, $120 worth to the acre	00	00
10.	Ground Bone (steamed), $120 worth to the acre	54	38
11.	Nitrate of Soda, $15 worth to the acre together with Muriate of Potash, $15 worth to the acre	11	34
12.	Ground Bone (steamed), $15 worth to the acre together with Nitrate of Soda, $15 worth to the acre	25	37

13.	Ground Bone (steamed), $15 worth to the acre together with Muriate of Potash, $15 worth to the acre	25	37
14.	Ground Bone (steamed), $10 worth to the acre together with Muriate of Potash, $10 worth to the acre, and Nitrate of Soda, $10 worth to the acre	22	18
15.	Ground Bone (steamed), $5 worth to the acre together with Nitrate of Soda, $5 worth to the acre, and Muriate of Potash, $20 worth to the acre	19	8
16.	Ground Bone (steamed), $5 worth to the acre together with Muriate of Potash, $5 worth to the acre, and Nitrate of Soda, $20 worth to the acre	19	39
17.	Ground Bone (steamed), $20 worth to the acre together with Muriate of Potash, $5 worth to the acre, and Nitrate of Soda, $5 worth to the acre	38	28


No. of the Experiment.
Comparative Weight of Crops.
10
7
4
1
17
2
12
13

COMPARATIVE WEIGHT OF CROPS.



No. 10. — *A Contribution to the History of the Use of Bark Bread.* By F. T. Dillingham, Instructor in Chemistry.

It has long been remarked by travellers that the inhabitants of northern countries, in times of dearth, are accustomed to use the inner bark of certain trees instead of, or as an addition to, grain for making bread. Most authorities, and noticeably the more trustworthy among them, lay special stress on the use of bark from coniferous trees; though, as will be seen below, mention is sometimes made of the inner bark of birch trees.

Thus Laing,* in his travels in Norway, remarks: —

> "Many of the dark, stern-looking pines were standing with all their branches dead. Stripped of their bark to make bread and burnished by the weather, resembling white marble, — mere ghosts of trees. The bread is made of the inner rind next to the wood, taken off in flakes like a sheet of foolscap paper, and is steeped or washed in warm water to clear off its astringent principle. It is then hung across a rope to dry in the sun, and looks exactly like sheets of parchment. When dry it is pounded into small pieces, mixed with corn [*i. e.* grain], and ground on the hand mill or quern. It is much more generally used than I supposed. There are districts in which the forests suffered very considerable damage in the years 1812 and 1814, when bad crops and the wars then raging reduced many to bark bread. The extended cultivation of the potato since that period has probably placed the inhabitants of the lower country beyond the necessity of generally resorting to it; but the Fjelde bönder use it more or less every year. It is not very unpalatable, nor is there any good reason for supposing it unwholesome, if well prepared; but it is very costly, for the value of the tree, which is left to perish on its roots, would buy a sack of flour, if the English market were open."

In his travels in Sweden the same author remarks: † —

> "I had heard of an earth found in the Fjelde being used as a substitute for meal, as well as the common substitute of ground pine bark. . . . Bark bread is at present in general use in all this part of the country. The new settlers have no other meal, and bake it very thick that it may hold together. It is acrid, dry; yet covered with plenty of butter it is eatable. The older settlers have at present rye meal to mix with it, half and half,

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* S. Laing, Journal of a Residence in Norway during the Years 1834, 1835 and 1836, London, 1859, pp. 219, 220.

† S. Laing, Tour in Sweden in 1838, London, 1839, pp. 181, 183.

and bake this mixture as thin as our (Scotch) oat cakes. This is so far from being uneatable, that prudent housekeepers in good circumstances use it to save their seed corn, even when grain is not dear; and the ruddy cheeks of the country girls prove that it is no unwholesome food, qualified no doubt as it is with plenty of butter and milk and hard work. The half and half, however, tastes strongly of timber and gets as hard as a board when kept long."

So, too, von Buch,* writing of Norway: —

"When the young and vigorous fir-trees are felled, to the great injury of the woods, the tree is stripped of its bark for its whole length; the outer part is carefully peeled from the bark; the deeper interior covering is then shaved off; and nothing remains but the innermost rind, which is extremely soft and white. It is then hung up several days in the air to dry, and afterwards baked in an oven; it is next beaten on wooden blocks, and then pounded as finely as possible in wooden vessels; but all this is not enough; the mass is yet to be carried to the mill and ground into coarse meal, like barley or oats. This meal is mixed with *hexel*(?), with threshed-out ears, or with a few moss seeds; and a bread of about an inch thickness is formed of this composition."

Clarke† refers to the matter repeatedly: —

"In this village the bread of the poor peasants was worse than any we had yet seen; it consisted of the inner bark of the fir-tree mixed with chaff and a very little barley. It seemed to us almost inconceivable that such bread could contain nourishment. We brought some of it to England; where it has remained ever since, unaltered, and in the same state in which it was offered to us for food."

"To add to the general wretchedness of the country, a greater dearth had prevailed during the former winter than the oldest person ever remembered. The people had saved themselves from starving by eating the bark bread, and a bread which they said they made of a kind of grass: this grass we afterwards found to be sorrel (*Rhumex acetosa*). The fir-bread had given to many of the inhabitants an unhealthy appearance; they found the sorrel bread upon the whole more salutary. The general effect of eating fir-bread is to produce a yellow, pale, and unhealthy countenance."‡

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* Von Buch cited by E. D. Clarke, Travels in Various Countries, 1824, **9.** 400, footnote.

† E. D. Clarke, Travels in Various Countries, 1824, **9.** 515.

‡ *Ibid.*, **10.** 473, 474.

On another page Clarke* quotes from Aubrey de la Motraye (see Travels of Aubrey de la Motraye, vol. 2, p. 288, London, 1732) as follows: “Travelling in Sweden, in the year 1718, we arrived upon the site of Tornea on the 19th of March. Scarcely a vestige of the town then remained, the Russians having burned it together with Umea and many other towns upon the coast. The inhabitants then used their *Missne bread* like some that we were afterwards compelled to eat.” And, as Aubrey de la Motraye most correctly describes the process, they made it from the rind of pine and fir trees in the following manner: —

> “They scrape the rough, crusty outside of the rind and clean off from the peel that part of it which is soft and white; this they dry, and with water- or hand-mills they grind it, and with the meal they make their bread in the same manner as we do with wheaten flour. There are some who at the same time dry and mix it with the powder of a certain herb, also dried up, which they call *Myessein*, and which is very plenty on the river side and in shallow waters; and others mix meal, made of wild oats which they gather in the woods.”

Clarke here goes on to say for himself that, —

> “The inhabitants of Tornea are become too fastidious now to feed on this primeval bread, for which the Swedish name is *Missne;* but the lapse of nearly a century has not banished it from the more northern parts of the country; and it is still found in seasons of scarcity even in Angermannland. We brought some of this bread to England, where it does not otherwise alter by keeping than that it is apt to become worm eaten, like an old board. In its original state, when we were forced by hunger to eat it, we never considered it as being worthy of the commendation which Linnaeus bestowed upon it.† The inhabitants of Ostro-Bothnia call it *Mäss;* and thus have preserved in the name of a kind of bread, which served as food among the ancestors of all the northern nations, an undoubted etymology of our word *mess.* The name, both among the Swedes and Fins, is derived from that of the plant used in making it, namely, the *Calla palustris.*”

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* Travels in Various Countries, 1824, 9. 352.

† Panis hic albus est, dulcis et gratissimus, praesertim recens. (Flora Lapponica, p. 250, Amst, 1737.)

One of the most convincing statements as regards the use of the bark of conifers is that given by Bayard Taylor.* In speaking of a famine in Finland, he says: "The younger children occupied themselves in peeling off the soft inner bark of the fir which they ate ravenously."

All of this evidence relating to the bark of coniferous trees consists so completely with the fact developed in this laboratory that the wood of coniferous trees contains much mannan, that it seemed possible that mannan in the bark might be the real source of nourishment.

There is, in fact, an instance where mannan is used as human food, namely, in Japan, where the root of *Conophallus konnjaku*, the dry matter of which is said to contain at least fifty per cent. of mannan and probably much more, is used in domestic economy.†

Both date stones and ivory nut contain large amounts of mannan; and it is a fact that date stones are an approved food for camels, and the coarse powder of the ivory nut has been used, even in this country, for feeding cattle. Both cattle and sheep eat the meal with great relish and fatten upon it.†

Recently Kellner has argued that in times of dearth fine spruce sawdust may well be used to replace straw in the rations of idle oxen, and may be regarded as possessing half the food value of straw.‡

Gordon Cumming § says: "Squirrels in Scotland do harm by eating the bark of coniferous trees within eight or ten feet of the summit whereby the trees are so enfeebled that gales break them, — even after the wound has healed. Young larch plantations and fir trees suffer much in early spring when the winter's store of nuts has been exhausted."

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* Bayard Taylor, Northern Travel, 1871, p. 93. Bayard Taylor was born in Chester County, Pennsylvania, in 1825, and was bred there on his father's farm. There is every reason to believe that his knowledge of trees, acquired through the usual training of an American boy of the period, must have been accurate. On general principles, it is to be presumed that it was superior to that of the ordinary literary writer of travels, and we are specifically assured by Taylor's biographers that "his early acquaintance with nature was both minute and general." It is said that in boyhood he profited not a little from the sympathy of one of his teachers — Martin — who took long walks with the lad and helped him to a fuller knowledge of tree and plant and flower and stone. (Life and Letters of Bayard Taylor. Edited by M. H. Taylor and H. E. Scudder, Boston, 1884, **1.** p. 12.)

† Bulletin of the Bussey Institution, 1897, **2.** 396, 397.

‡ Central Blatt für Agricultur Chemie, 1895, **24.** 164.

§ C. F. Gordon Cumming, "Memories," Edinburgh and London, 1904, pp. 389–392.

At this point may be mentioned the habit of many animals, such as mice, rabbits, beavers, and porcupines, of feeding upon the bark of trees in preference to the wood.*

As was said previously, some writers have made special mention of the bark of the birch, as well as of the elm and poplar.

Thus Clarke says: "The bark of the birch is serviceable to the natives in various ways; mingled with barley meal, it constitutes a part of their food.† . . . The bread of this family was full of chaff and of the bark of the birch tree; it was only when stewed in butter that we were able to swallow it, and even then with difficulty." ‡

Again he says: "Upon our return we found our boatmen at their scanty meal which always consisted of the same diet; nor did they seem desirous either to add to their food or to alter it. This consisted only of biscuit made of the inner bark of the birch tree, chopped straw, and a little rye; which they washed down with a beverage, swallowed greedily by quarts at a time, of coagulated sour milk." § In a note Clarke adds, "Sometimes we heard that the biscuit was made with *birch* and sometimes with *fir* bark."

Bishop Pontoppidan ‖ says that elm bark dried, ground, and mixed with meal is used as food by the peasants for themselves and their animals.

In referring to the "cottonwood" (*Populus monolifera*) here in America, Maud Going ¶ says: —

> "Between the bark and the wood there is, in the spring, a sweet, milky juice of which the Indians are very fond. As one who had been educated in the East said, — it is their ice cream, and they frequently strip the bark and scrape the trunk in order to procure it. Horses also are fond of this sweet juice, and in

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* Bulletin of the Bussey Institution, 1897, **2.** 390.

† E. D. Clarke, Travels in Various Countries, 1824, **9.** 540,

‡ *Ibid.*, **9.** 550.

§ *Ibid.*, **9.** 400.

‖ K. E. Tyler, The Story of a Scandinavian Summer, New York, 1881, p. 229.

¶ Maud Going. With the Trees, 1903, p. 171.

seasons when the grass has been burned off, or is otherwise scarce, the Indian ponies sometimes resort to the small twigs and bark of the cottonwood to sustain life. In extreme cases their owners have sometimes been driven to the same shift."

In the hope of throwing more light on the apparent superiority of the bark of conifers, I have tested several kinds of barks chemically for mannan in the same way that the woods of various trees, roots, and fruits have been examined, in former years, in this laboratory,* the idea being to ascertain how large a proportion of mannan is contained in the barks as compared with the quantities contained in the woods proper.

At Durham, New Brunswick, during the summer of 1905, I availed myself of an excellent opportunity to collect several samples of inner bark of the fir (*Picea balsamifera*), pine (*Pinus strobus*), spruce (*Picea nigra*), and larch (*Larix Americana*), as well as of the gray birch (*Betula populifolia*) and poplar (*Populus tremuloides*).

In the case of the conifers, young, vigorous trees were selected. They were from 2 to 4½ inches in diameter at the butt. The bark from the trunk wood, and in one instance from the larger limbs, was collected. The outer bark was first removed; the inner bark was then carefully peeled from the wood in as large pieces as possible. These pieces were air dried in a warm room, and kept in a dry condition until the time of using.

The sample of poplar bark was collected in the same manner. The tree measured 3½ inches in diameter at the lower end. Bark from the trunk wood only was taken.

In case of the birch one limb, about 2½ inches in diameter, of a forked tree, was selected. The rather thick, greenish inner bark was taken as the sample.

In preparing each sample for use the material was cut into small pieces and ground to a fine meal in a hand-mill.

The method of procedure was similar to that followed previously.† A quantity of the powdered material equal to about twenty grams of the substance reckoned as dry at 100° C. was boiled in a flask, with a reflux condenser, for three hours, with ten times as many cubic centimeters of hydrochloric acid of five per cent. as there had been taken grams of the air-dried material. The contents of the flask were filtered after having stood over night, the residue was washed with ten cubic centimeters of water, and the washings were added to the filtrate. Ten cubic centimeters of the liquid were neutralized to slight alkalinity with a solution of sodium hydroxide (1–8), and then rendered faintly acid with a few drops of a 5 per cent. solution of hydrochloric acid. This unevaporated liquid was next divided into several one cubic centimeter portions. One of these portions was tested directly by the addition to it of twelve drops of a solution of phenyl-hydrazine acetate. [This reagent was prepared by adding 2 c.c. of glacial acetic acid to 1 c.c. of phenyl-hydrazine, and shaking thoroughly after the addition of 10 c.c. of water.] Other one cubic centimeter portions of the liquid were diluted with one cubic centimeter and two cubic centimeters of water, respectively, and to each of these diluted portions twelve drops of the solution of phenyl-hydrazine acetate were added. The precipitates which formed were examined under the microscope, but in no case was mannose-hydrazone detected in any of these unevaporated liquids.

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* Bulletin of the Bussey Institution, 1903, **3**. 47.

† Bussey Bulletin, 1903, **3**. 47.

The remainder of the filtrate (and washings) from hydrolysis was neutralized to slight alkalinity with a solution of sodium hydroxide (1–8), and then made faintly acid by the addition of a small quantity of a 5 per cent. solution of hydrochloric acid. The nearly neutral liquid was evaporated on a water-bath to soft dryness. The residue was warmed with ten cubic centimeters of water, allowed to cool, and the liquid filtered. The residue was washed with one cubic centimeter of water; the washings were added to the filtrate and thoroughly mixed with it. This evaporated liquor was tested in the following manner: It was divided into several one cubic centimeter portions. To one of these portions sixteen drops of a solution of phenyl-hydrazine acetate were added directly. Other one cubic centimeter portions were diluted with one, two, and three cubic centimeters of water, respectively, and to each of these diluted portions sixteen drops of the solution of phenyl-hydrazine acetate were added. The precipitates which formed were examined under the microscope within two hours after they had fallen, and again on the next day. If no mannose-hydrazone could be detected at the end of this time it was considered to be absent.

Nine samples of inner bark were treated in the manner just described. The results obtained appear in the following table. (The word "yes" indicates that mannose-hydrazone was detected. The word "no" indicates that mannose-hydrazone was not detected.)

Kind of Material.	Unevaporated Liquid			Evaporated Liquid			
	1 c.c. alone.	1 c.c. + 1 c.c. water.	1 c.c. + 2 c.c. water.	1 c.c. alone.	1 c.c. + 1 c.c. water.	1 c.c. + 2 c.c. water.	1 c.c. + 3 c.c. water.
Inner bark of Fir, cut July 5 and 6, 1905	No	No	No	Yes	No	No	No
Inner bark of Spruce, cut July 7, 1905	No	No	No	No	No	No	No
Inner bark of Pine, cut July 7, 1905 .	No	No	No	Yes	No	No	No
Inner bark of Fir, cut July 31, 1905 .	No	No	No	Yes	Yes	No	No
Inner bark of Spruce, cut Aug. 7, 1905	No	No	No	Yes	Tr.	No	No
Inner bark of Larch, cut Aug. 9, 1905 .	No	No	No	Yes	No	No	No
Inner bark of Pine, cut Aug. 12, 1905 .	No	No	No	Yes	Yes	No	No
Inner bark of Poplar, American aspen, cut Aug. 10, 1905	No	No	No	No	No	No	No
Inner bark of Gray Birch, cut Aug. 11, 1905	No	No	No	No	No	No	No

On comparing these results obtained with barks with those previously obtained in this laboratory from woods proper,* it will be seen that the barks apparently contain decidedly smaller quantities of mannan than is contained in the actual woods. Roughly speaking, it may be said that at the time of year when mannan is present in largest proportion in the wood, there is, according to our method of estimation, only about one fourth as much of it in the bark; and the question naturally arises if mannan is really the source of nourishment in bark bread, why did not the Scandinavians grind *wood* to a powder in the manner described as well as, or rather than, the bark?

It is noteworthy that while it had been found previously that the quantity of mannan in pine wood is apparently largest in

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* Bulletin of the Bussey Institution, 1903, **3.** 50.

August, so, here, the quantity of mannan in a sample of inner bark of pine collected in August was evidently larger than in a sample collected in the preceding month. The same fact is evident in the case of the samples of fir bark, and apparently in the case of the spruce bark also. Hence the suggestion is offered that the amount of mannan in bark increases relatively with the increase of mannan in wood proper. More tests are necessary to confirm this view.

No mannan was detected in samples of the inner bark of the gray birch (*Betula populifolia*) and of the poplar (*American aspen*, *Populus tremuloides*). Previous work in this laboratory with the wood of these trees had failed to detect the presence of mannan therein.*

Contrary to expectations, the results of this investigation failed to show any such preponderance of mannan in the inner bark of conifers as might serve to explain fully the use of the bark as food. It is not to be denied, as yet, that the mannan in bark bread may be of real importance for human sustenance; but, to all appearance, further work will be needed to account completely for the physiological significance of this kind of food.

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* Bulletin of the Bussey Institution, 1902, **3**. 34, 35.

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